

APS
9/29/97

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(FILE 'USPAT' ENTERED AT 09:40:10 ON 29 SEP 1997)

	E METZ, JAMES/IN
L1	4 S E4
	E LARDIZABAL, KATHRYN/IN
L2	1 S E4
	E LASSNER, MICHAEL/IN
L3	4 S E5
L4	6 S L1-L3
L5	6998 S FATTY/AB
L6	4 S L4 AND L5
L7	196 S (CONDENSING (W) ENZYME? OR SYNTHASE?) (P) REDUCTASE?
L8	8 S L5 AND L7
L9	4 S L8 NOT L6

=> :pt 16 cit,ab 1-4

1. 5,445,947, Aug. 29, 1995, Jojoba wax biosynthesis gene; **James G. Metz**, et al., 435/69.1, 71.2, 134, 172.3, 419; 536/23.2, 23.6; 800/200, 205, 255, DIG.17 :IMAGE AVAILABLE:

US PAT NO: 5,445,947 :IMAGE AVAILABLE:

L6: 1 of 4

ABSTRACT:

By this invention, a partially purified **fatty** acyl-CoA: **fatty** alcohol acyltransferase (wax synthase) is provided, wherein said protein is active in the formation of a wax ester from **fatty** alcohol and **fatty** acyl substrates. Of special interest is a jojoba embryo wax synthase having an apparent molecular mass of approximately 57 kD. Also considered are amino acid and nucleic acid sequences obtainable from wax synthase proteins and the use of such sequences to provide transgenic host cells capable of producing wax esters.

2. 5,411,879, May 2, 1995, Fatty acyl reductases; Michael R. Pollard, et al., 435/190, 189; 530/377 :IMAGE AVAILABLE:

US PAT NO: 5,411,879 :IMAGE AVAILABLE:

L6: 2 of 4

ABSTRACT:

By this invention, a solubilized seed-plant **fatty** acyl reductase protein is provided, wherein said protein is active in the formation of a **fatty** alcohol from a **fatty** acyl substrate. Of special interest is a jojoba embryo reductase protein having a molecular mass of about 32 kD or about 47 kD and sequences obtainable therefrom. Also considered are amino acid and nucleic acid sequences obtainable from such **fatty** acyl reductases.

3. 5,403,918, Apr. 4, 1995, Fatty acyl reductase; **James G. Metz**, 530/379; 435/189; 530/344 :IMAGE AVAILABLE:

US PAT NO: 5,403,918 :IMAGE AVAILABLE:

L6: 3 of 4

ABSTRACT:

By this invention, a partially purified seed-plant **fatty** acyl reductase protein is provided, wherein said protein is active in the formation of a **fatty** alcohol from a **fatty** acyl substrate. Of special interest are jojoba embryo reductase proteins having molecular mass of about 54 and 52 kD and sequences obtainable therefrom. Also considered are amino acid and nucleic acid sequences obtainable from such **fatty** acyl reductases.

4. 5,370,996, Dec. 6, 1994, Fatty acyl reductases; **James G. Metz**, et al., 435/69.1, 70.1, 71.2, 134, 172.3, 252.3, 252.33, 320.1, 419; 536/23.2, 23.6 :IMAGE AVAILABLE:

US PAT NO: 5,370,996 :IMAGE AVAILABLE:

L6: 4 of 4

ABSTRACT:

By this invention, a partially purified seed-plant **fatty** acyl reductase protein is provided, wherein said protein is active in the formation of a **fatty** alcohol from a **fatty** acyl substrate. Of special interest are jojoba embryo reductase proteins having molecular mass of about 54 and 52 kD and sequences obtainable therefrom. Also

BSum(8)

considered are amino acid and nucleic acid sequences obtainable from such **fatty** acyl reductases, which sequences may be used for preparation of recombinant constructs useful for expression of reductase in host cells, which results in the production of **fatty** alcohols in said cells.

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Dialog
9/29/97

Set	Items	Description
S1	718	E3,E10,E11
S2	215	E3,E7
S3	6	AU="METZ JG"
S4	2	AU="METZ J.G."
S5	1	AU="METZ, JAMES GEORGE"
S6	940	S1-S5
S7	4	AU=LARDIZABAL K
S8	6	AU=LARDIZABAL K D
S9	1	AU=LARDIZABAL KD
S10	1	AU=LARDIZABAL, K.
S11	1	AU=LARDIZABAL, K.D.
S12	13	E3-E5, E14-E15
S13	53	E3,E5-E7,E14-E15,E17
S14	984	S1-S13
S15	428	FATTY AND (CONDENSING (W) ENZYM? OR SYNTHASE?) AND REDUCTA- SE?
S16	1	S14 AND S15
S17	2953	WAX (3N) ESTER?
S18	1	S15 AND S17
S19	0	S18 NOT S16
S20	299	FATTY (W) (ACID? OR ACYL) (5N) ((CONDENSING (W) ENZYM? OR - SYNTHASE?) AND REDUCTASE?)
S21	2501783	PLANT OR PLANTS
S22	46	S20 AND S21
S23	30	RD (unique items)

16/7/1 (Item 1 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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010409548

WPI Acc No: 95-310894/199540

DNA construct expressing jojoba wax **synthase** and transformed
Brassica cells - useful for producing wax ester(s) for use in
pharmaceuticals and cosmetics, etc

Patent Assignee: CALGENE INC (CALJ)

Inventor: **LARDIZABAL K D**; **LASSNER M W**; **METZ J G**

Number of Countries: 001 Number of Patents: 001

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
US 5445947	A	19950829	US 91796256	A	19911120	C12P-001/04	199540 B
			US 92933411	A	19920821		
			WO 92US9863	A	19921113		
			US 9366299	A	19930520		

Priority Applications (No Type Date): US 9366299 A 19930520; US 91796256 A
19911120; US 92933411 A 19920821; WO 92US9863 A 19921113

Patent Details:

Patent	Kind	Lan	Pg	Filing Notes	Application	Patent
US 5445947	A		50	CIP of	US 91796256	
				CIP of	US 92933411	
				CIP of	WO 92US9863	

Abstract (Basic): US 5445947 A

A recombinant DNA construct is new which comprises a nucleic acid sequence (I) encoding the 524 or 521 amino acid proteins and a heterologous DNA sequence (II) not naturally associated with (I). Also new is a Brassica plant cell which contains a construct as above which encodes a protein that is heterologous to the host, under control of a promoter functional in the host cell.

USE - (I) encodes **fatty** acyl-CoA: **fatty** alcohol O-acyltransferase ('wax **synthase**') from jojoba (*Simmondsia chinensis*). This enzyme is involved in biosynthesis of wax esters from **fatty** alcohols and **fatty** acyl substrates. (I) is used for prodn. of recombinant wax **synthase** or to isolate related sequences from other organisms, while the enzyme is used to produce wax esters in cells that do not normally produce it (partic. when the cells are also engineered to express a **fatty** acyl **reductase**). Wax esters are useful in pharmaceuticals, cosmetics, detergents, plastics and lubricants.

Dwg.0/3

Derwent Class: B04; D16; D21; P14

International Patent Class (Main): C12P-001/04

International Patent Class (Additional): A01M-001/00; C12N-015/05;

? t s23/7/all

23/7/1 (Item 1 from file: 5)
DIALOG(R) File 5:BIOSIS PREVIEWS(R)
(c) 1997 BIOSIS. All rts. reserv.

13549156 BIOSIS Number: 99549156

A **fatty acid synthase** gene in *Cochliobolus carbonum* required for production of HC-toxin, cyclo(D-prolyl-L-alanyl-D-alanyl-L-2-amino-9,10-epoxi-8-oxodecanoyl)

Ahn J-H; Walton J D

DOE-Plant Res. Lab., Mich. State Univ., East Lansing, MI 48824, USA

Molecular Plant-Microbe Interactions 10 (2). 1997. 207-214.

Full Journal Title: Molecular Plant-Microbe Interactions

ISSN: 0894-0282

Language: ENGLISH

Print Number: Biological Abstracts Vol. 103 Iss. 012 Ref. 172584

The fungal maize pathogen *Cochliobolus carbonum* produces a phytotoxic and cytostatic cyclic peptide, HC-toxin, of structure cyclo(D-prolyl-L-alanyl-D-alanyl-L-Aeo), in which Aeo stands for 2-amino-9,10-epoxi-8-oxodecanoic acid. Here we report the isolation of a gene, TOXC, that is present only in HC-toxin-producing (Tox2+) fungal strains. TOXC is present in most Tox2+ strains in three functional copies, all of which are on the same chromosome as the gene encoding HC-toxin synthetase. When all copies of TOXC are mutated by targeted gene disruption, the fungus grows and sporulates normally in vitro but no longer makes HC-toxin and is not pathogenic, indicating that TOXC has a specific role in HC-toxin production and hence virulence. The TOXC mRNA is 6.5 kb and the predicted product has 2,080 amino acids and a molecular weight of 233,000. The primary amino acid sequence is highly similar (45 to 47% identity) to the beta subunit of **fatty acid synthase** from several lower eukaryotes, and contains, in the same order as in other beta subunits, domains predicted to encode acetyl transferase, enoyl reductase, dehydratase, and malonyl-palmityl transferase. The most plausible function of TOXC is to contribute to the synthesis of the decanoic acid backbone of Aeo.

23/7/2 (Item 2 from file: 5)
DIALOG(R) File 5:BIOSIS PREVIEWS(R)
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13415197 BIOSIS Number: 99415197

Decarboxylation of malonyl-(acyl carrier protein) by 3-oxoacyl-(acyl carrier protein) **synthases** in **plant fatty acid** biosynthesis

Winter E; Brummel M; Schuch R; Spener F

Inst. fuer Chemo- und Biosensorik, Mendelsr. 7, D-48149 Muenster, Germany

Biochemical Journal 321 (2). 1997. 313-318.

Full Journal Title: Biochemical Journal

ISSN: 0264-6021

Language: ENGLISH

Print Number: Biological Abstracts Vol. 103 Iss. 006 Ref. 087340

In order to identify regulatory steps in fatty acid biosynthesis, the influence of intermediate 3-oxoacyl-(acyl carrier proteins) (3oxoacyl-ACPs) and end-product acyl-ACPs of the **fatty acid synthase** reaction on the condensation reaction was investigated in vitro, using total **fatty acid synthase** preparations and purified

3-oxoacyl-ACP synthases (KASs; EC 2.3.1.41) from *Cuphea lanceolata* seeds. KAS I and II in the **fatty acid synthase** preparations were assayed for the elongation of octanoyl- and hexadecanoyl-ACP respectively, and the accumulation of the corresponding condensation product 3-oxoacyl-ACP was studied by modulating the content of the reducing equivalents NADH and NADPH. Complete omission of reducing equivalents resulted with either KAS in the abnormal synthesis of acetyl-ACP from malonyl-ACP by decarboxylation reaction. Supplementation with NADPH or NADH, separately or in combination with recombinant 3-oxoacyl-ACP reductase (EC 1.1.1.100), led to a decrease in the amount of acetyl-ACP and a simultaneous increase in elongation products. This demonstrates that the accumulation of 3-oxoacyl-ACP inhibits the condensation reaction on the one hand, and induces the decarboxylation of malonyl-ACP on the other. By carrying out similar experiments with purified enzymes, this decarboxylation was attributed to the action of KAS. Our data point to a regulatory mechanism for the degradation of malonyl-ACP in **plants** which is activated by the accumulation of the **fatty acid synthase** intermediate 3-oxoacyl-ACP.

23/7/3 (Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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11032311 BIOSIS Number: 97232311

The mechanism of inhibition of **fatty acid synthase** by the herbicide diflufenican

Ashton I A; Abulnaja K O; Pallett K E; Cole D J; Harwood J L
Dep. Biochem., Univ. Wales College Cardiff, Cardiff CF1 1ST, UK
Phytochemistry (Oxford) 35 (3). 1994. 587-590.

Full Journal Title: Phytochemistry (Oxford)

ISSN: 0031-9422

Language: ENGLISH

Print Number: Biological Abstracts Vol. 097 Iss. 010 Ref. 149424

The bleaching herbicide diflufenican (N-(2,4-difluorophenyl)-2-(3-(trifluoromethyl)phenoxy)-3-pyridinecarboxamide) has been shown to inhibit **plant fatty acid synthase**. The mechanism of this inhibition was studied further by measuring the activities of the **reductase** components of the Type II **fatty acid synthase** complexes from *Escherichia coli* and avocado (*Persea americana*) mesocarp. Diflufenican had no effect on beta-ketoacyl-ACP reductase activity, but competitively inhibited both NADH- and NADPH-dependent enoyl-ACP reductases. This result suggests that chemicals based on the diflufenican structure may be potential herbicides by virtue of their inhibition of fatty acid synthesis.

23/7/4 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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10090212 BIOSIS Number: 95090212

PARTIAL PURIFICATION AND CHARACTERIZATION OF A POLYKETIDE BIOSYNTHETIC ENZYME 6-HYDROXYMELLEIN SYNTHASE IN ELICITOR-TREATED CARROT CELL EXTRACTS

KUROSAKI F; ITOH M; KIZAWA Y; NISHI A

FAC. PHARMACEUTICAL SCI., TOYAMA MED. PHARMACEUTICAL UNIV., SUGITANI, TOYAMA 930-01, JPN.

ARCH BIOCHEM BIOPHYS 300 (1). 1993. 157-163. CODEN: ABBIA

Full Journal Title: Archives of Biochemistry and Biophysics

Language: ENGLISH

6-Hydroxymellein synthase, an induced polyketide biosynthetic enzyme in carrot cell extracts, was purified about 240-fold and its properties were compared with those of fatty acid synthetases. Synthetic activity of 6-hydroxymellein was inhibited in the presence of sulphydryl reagents; however, cerulenin, a well-known inhibitor of fatty acid synthetases,

showed no inhibitory activity to the enzyme. Biosynthesis of 6-hydroxymellein includes an NADPH-dependent ketoreduction, and, in this reaction, the 4-pro-S-hydrogen of NADPH was specifically transferred to the compound. On the basis of stereochemical analyses of the biosynthetic process, it was concluded that the product of the ketoreduction is an optically active alcohol of R configuration. These stereo-specificities of the reduction process are identical to those of .beta.-ketoacyl **reductase** in **fatty acid** biosynthesis which are considered to be conserved in all organisms. The synthetic rate of 6-hydroxymellein was markedly reduced when the assay was carried out with deuterium-labeled NADPH. The observed isotope effect on the catalytic rate (kH/kD) was 5.20, suggesting that this ketoreduction is one of the rate-limiting processes in 6-hydroxymellein synthesis. More than 85% of the synthetic activity was found in the soluble fraction of carrot cells, and, unlike in fatty acid synthetases in higher **plants**, organelle-localizing activity was not observed.

23/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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7771229 BIOSIS Number: 90139229

THE MULTIFUNCTIONAL 6 METHYLSALICYLIC ACID SYNTHASE GENE OF
PENICILLIUM-PATULUM ITS GENE STRUCTURE RELATIVE TO THAT OF OTHER POLYKETIDE
SYNTHASES

BECK J; RIPKA S; SIEGNER A; SCHILTZ E; SCHWEIZER E
LEHRSTUHL FUER BIOCHEMIE DER UNIVERSITAET ERLANGEN-NUERNBERG,
STAUDTSTRASSE 5, D-8520 ERLANGEN, FRG.

EUR J BIOCHEM 192 (2). 1990. 487-498. CODEN: EJBCA

Full Journal Title: European Journal of Biochemistry

Language: ENGLISH

6-Methylsalicylic acid synthase (MSAS) from *Penicillium patulum* is a homomultimer of a single, multifunctional protein subunit. The enzyme is induced, at the transcriptional level, during the end of the logarithmic growth phase. After approximately 150-fold purification, a homogeneous enzyme preparation was obtained exhibiting, upon SDS gel electrophoresis, a subunit molecular mass of 188 kDa. By immunological screening of a genomic *P. patulum* DNA expression library, the MSAS gene together with its flanking sequences was isolated; 7131 base pairs of the cloned genomic DNA were sequenced. Within this sequence the MSAS gene was identified as a 5322-bp-long open reading frame coding for a protein of 1774 amino acids and 190731 Da molecular mass. Transcriptional initiation and termination sites were determined both by primer extension studies and from cDNA sequences specially prepared for the 5' and 3' portions of the gene. The same cDNA sequences revealed the presence of a 69-bp intron within the N-terminal part of the MSAS gene. The intron contains the canonical GT and AG dinucleotides at its 5'- and 3'-splice junctions. An internal TACTGAC sequence, resembling the TACTAAC consensus element of *Saccharomyces cerevisiae* introns is suggested to represent the branch point of the lariat splicing intermediate. When compared to other known polyketide synthases, distinct amino acid sequence similarities of limited lengths were observed with some, though not all, of them. A comparatively low degree of similarity was detected to the yeast and *Penicillium* FAS or to the **plant** chalcone and resveratrol synthases. In contrast, a significantly higher sequence similarity was found between MSAS and the rat **fatty acid synthase**, especially at their transacyl **reductase**, 2-oxoacylase, 2-oxoacyl synthase and acyl carrier protein domains. Besides several dissimilar, interspersed regions probably coding for MSAS- and FAS-specific functions, the sequential order of the similar domains was colinear in both enzymes. The low similarity between the two *P. patulum* polyketide synthases, MSAS and FAS, possibly supports a convergent rather than a divergent evolution of both multienzyme proteins.

23/7/6 (Item 6 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
(c) 1997 BIOSIS. All rts. reserv.

7553387 BIOSIS Number: 39065994
ENZYMOLGY AND MOLECULAR BIOLOGY OF **PLANT** LIPID BIOSYNTHESIS
SLABAS A R
UNILEVER, SHARNBROOK.
1990 ANNUAL MEETING OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY. J EXP BOT 41
(SUPPL.). 1990. P8-2. CODEN: JEBOA
Language: ENGLISH

23/7/7 (Item 7 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
(c) 1997 BIOSIS. All rts. reserv.

6251239 BIOSIS Number: 35116760
2 OXOACYL ACYL CARRIER PROTEIN **REDUCTASE** A COMPONENT OF **PLANT**
FATTY ACID SYNTHASE
SHELDON P S; SAFFORD R; SLABAS A R; KEKWICK R G O
DEP. BIOCHEMISTRY, UNIV. BIRMINGHAM, P.O. BOX 363, BIRMINGHAM B15 2TT,
U.K.
624TH MEETING OF THE BIOCHEMICAL SOCIETY, DUBLIN, IRELAND, SEPTEMBER
22-25, 1987. BIOCHEM SOC TRANS 16 (3). 1988. 392-393. CODEN: BCSTB
Language: ENGLISH

23/7/8 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abstracts Online
(c) 1997 UMI. All rts. reserv.

01351412 ORDER NO: AAD94-11760
ISOLATION AND CHARACTERIZATION OF A SYNTHETIC GENE AND A GENOMIC CLONE
ENCODING ACYL CARRIER PROTEIN FROM ESCHERICHIA COLI
Author: RAWLINGS, MERRIANN
Degree: PH.D.
Year: 1993
Corporate Source/Institution: UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
(0090)
Adviser: J. E. CRONAN, JR.
Source: VOLUME 54/11-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 5519. 92 PAGES

Acyl carrier protein (ACP) is a required cofactor for the synthesis and subsequent metabolism of fatty acids in Escherichia coli. Previous work has suggested that DNA segments encoding ACP were somehow toxic to E. coli. To investigate this possibility, a synthetic gene encoding ACP was assembled using a novel λ lacZ α -complementation test. When provided with the sequences necessary for transcription and translation, the gene was expressed at high levels. Despite the already functional excess of ACP in wild-type cells its overexpression was indeed lethal, resulting in a decreased cellular growth rate and a precipitous drop in cell viability. One of the most obvious differences between ACP overproducing and wild-type strains was the accumulation of apo-ACP by the former cells. Normally apo-ACP is not detected in vivo. A genomic clone encoding ACP has also been isolated and sequenced. The ACP gene (called acpP) was located on the genetic map between fabF and fabD which encode two **fatty acid** biosynthetic enzymes, 3-ketoacyl-ACP **synthase** II and malonyl CoA-ACP transacylase, respectively. An open reading frame between acpP and fabD encodes a 26.5-kDa protein that has significant sequence identity (>40%) with a **plant** 3-ketoacyl-ACP reductase and thus is believed to encode the same enzyme in E. coli. This gene (called fabG) is cotranscribed with acpP. Thus, the gene encoding ACP is located within a cluster of fatty acid biosynthetic genes.

23/7/9 (Item 2 from file: 35)
DIALOG(R)File 35:Dissertation Abstracts Online
(c) 1997 UMI. All rts. reserv.

01120867 ORDER NO: AAD90-25382
PURIFICATION AND CHARACTERIZATION OF ENOYL-ACP REDUCTASE FROM EUGLENA GRACILIS

Author: TUCKER, MARGIE MCGEE
Degree: PH.D.
Year: 1990
Corporate Source/Institution: EAST TENNESSEE STATE UNIVERSITY (0069)
CHAIRMAN: MARY LOU ERNST-FONBERG
Source: VOLUME 51/04-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 1808. 209 PAGES

Enoyl-(acyl-carrier-protein) reductase was purified from the phytoflagellate *Euglena gracilis*. Its purification employed DEAE-Sephacel chromatography, Matrex Orange chromatography, and affinity chromatography using acyl carrier protein (ACP) covalently bound to Sepharose as the affinity ligand. Matrex Orange chromatography resolved two different enoyl-ACP reductases having different characteristics. *Euglena gracilis* appears to resemble higher **plants** in the possession of two isoforms of this enzyme.

Antibodies specific for the cofactor binding site of NADP (H)-requiring dehydrogenases were obtained. They were isolated from a polyclonal population of antibodies directed against yeast glucose-6-phosphate dehydrogenase by affinity chromatography using chicken liver malic enzyme as the affinity ligand. The affinity purified antibodies were covalently bound to Sepharose. Glucose-6-phosphate dehydrogenase and malic enzyme were both bound by the antibody column and were eluted by their cofactor, NADP, identifying the site of recognition of the enzymes by the antibodies as the cofactor binding site. The utility of this antibody affinity column was demonstrated by its ability to bind enoyl-ACP reductase, which was eluted by its cofactor, NADPH.

Preliminary studies of the *E. gracilis* **fatty acid synthase** (FAS) genes were undertaken using the plasmid pFAS4 (Witkowski et al., 1987), which contains a cDNA insert to part of the rat liver FAS mRNA and was a gift of Dr. Stuart Smith. The insert was cleaved with KpnI and PstI to generate probes specific for the ketoreductase, ACP, and thioesterase domains of the FAS. DNA from wild type *E. gracilis* and from a mutant, W₁₀S₁₀ML, which lacks chloroplast DNA, was subjected to field inversion gel electrophoresis and the DNA alkaline-blotted onto Nylon membranes. Hybridization of the three probes to the DNA was performed; all three probes hybridized to nuclear DNA, but none of the three hybridized to chloroplast DNA. The three probes also hybridized to a band which was neither nuclear nor chloroplast DNA. This DNA, which was larger than the chloroplast genome, may represent *E. gracilis* mitochondrial DNA sequences.

23/7/10 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
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10218606 EMBASE No: 97016729

Soluble and membrane bound components of **plant** lipid synthesis
COMPOSES MEMBRANAIRES ET SOLUBLES IMPLIQUES DANS LA SYNTHÈSE DES LIPIDES
CHEZ LES PLANTES

Slabas A.R.; Brown A.P.; Rafferty J.B.; Rice D.W.; Baldock C.; Kroon J.T.M.; Simon W.; Stuitje A.R.; Brough C.L.

A.R. Slabas, Lipid Molecular Biology Group, Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE United Kingdom
Comptes Rendus de l'Academie des Sciences - Serie III (France) , 1996,

319/11 (1043-1047) CODEN: CRASE ISSN: 0764-4469
DOCUMENT TYPE: Journal
LANGUAGES: English SUMMARY LANGUAGES: French; English
NUMBER OF REFERENCES: 13

Enoyl ACP reductase (ENR) catalyzes the NADH dependent reduction of trans enoyl ACP to form saturated acyl ACPs, it is an essential component of the multisubunit type II fatty acid synthetase which is highly expressed in a temporal specific manner in seeds. The enzyme has been purified from rape, extensively sequenced its cDNA cloned, and the protein overexpressed and crystallized. The complete 3-dimensional structure of the enzyme has been determined at 1.9 Å. Difference Fourier analysis has shown that crotonyl ACP is a better substrate than crotonyl CoA as the latter also binds to the NADH pocket of the enzyme and thereby acts as an enzyme inhibitor. The potential active site has been identified from the position of conserved residues and by the location of the position of the nicotinamide ring of NADH. In addition extensive structural similarity has been found between ENR and the 3α-hydroxysteroid dehydrogenase. This has provided insights into the catalytic mechanisms which are being tested by site directed mutagenesis. In an attempt to gain insight into membrane bound enzymes of lipid biosynthesis we have employed a complementation cloning technique in *E. coli* to isolate the membrane bound 2-acyltransferase which has defied conventional purification techniques. In the first instance we cloned a 2-acyltransferase (2-AT) from maize and more recently we have cloned two 2-acyltransferases from *Limnanthes douglasii*. One of these shows distinct substrate specificity differences to the *E. coli* 2-AT. Introduction of the cDNA encoding this 2-AT into a high erucic acid rape line has allowed the synthesis of trierucin in the transgenic seed. Analysis of the transgenes and other acyltransferases is in progress.

23/7/11 (Item 2 from file: 73)
DIALOG(R) File 73:EMBASE
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9894257 EMBASE No: 96075274

Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*

Brown D.W.; Yu J.-H.; Kelkar H.S.; Fernandes M.; Nesbitt T.C.; Keller N.P.; Adams T.H.; Leonardo T.J.

Department of Biology, Clark University, 950 Main Street, Worcester, MA 01610 USA

Proceedings of the National Academy of Sciences of the United States of America (USA), 1996, 93/4 (1418-1422) CODEN: PNAS ISSN: 0027-8424

LANGUAGES: English SUMMARY LANGUAGES: English

Sterigmatocystin (ST) and the aflatoxins (AFs), related fungal secondary metabolites, are among the most toxic, mutagenic, and carcinogenic natural products known. The ST biosynthetic pathway in *Aspergillus nidulans* is estimated to involve at least 15 enzymatic activities, while certain *Aspergillus parasiticus*, *Aspergillus flavus*, and *Aspergillus nomius* strains contain additional activities that convert ST to AF. We have characterized a 60-kb region in the *A. nidulans* genome and find it contains many, if not all, of the genes needed for ST biosynthesis. This region includes *verA*, a structural gene previously shown to be required for ST biosynthesis, and 24 additional closely spaced transcripts ranging in size from 0.6 to 7.2 kb that are coordinately induced only under ST-producing conditions. Each end of this gene cluster is demarcated by transcripts that are expressed under both ST-inducing and non-ST-inducing conditions. Deduced polypeptide sequences of regions within this cluster had a high percentage of identity with enzymes that have activities predicted for ST/AF biosynthesis, including a polyketide **synthase**, a **fatty acid synthase** (alpha and beta subunits), five monooxygenases, four dehydrogenases, an esterase, an O-methyltransferase, a reductase, an oxidase, and a zinc cluster DNA binding protein. A revised system for naming the genes of the ST pathway is presented.

23/7/12 (Item 3 from file: 73)
DIALOG(R) File 73:EMBASE
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9832219 EMBASE No: 96009237

Structure and function of fas-1A, a gene encoding a putative fatty acid synthetase directly involved in aflatoxin biosynthesis in *Aspergillus parasiticus*

Mahanti N.; Bhatnagar D.; Cary J.W.; Joubran J.; Linz J.E.

Food Science/Human Nutrition Dept., Michigan State University, East Lansing, MI 48824 USA

Applied and Environmental Microbiology (USA) , 1996, 62/1 (191-195)

CODEN: AEMID ISSN: 0099-2240

LANGUAGES: English SUMMARY LANGUAGES: English

A novel gene, fas-1A, directly involved in aflatoxin B1 (AFB1) biosynthesis, was cloned by genetic complementation of an *Aspergillus parasiticus* mutant strain, UVM8, blocked at two unique sites in the AFB1 biosynthetic pathway. Metabolite conversion studies localized the two genetic blocks to early steps in the AFB1 pathway (nor-I and fas-1A) and confirmed that fas-1A is blocked prior to nor-1. Transformation of UVM8 with cosmid NorA and NorB restored function in nor-1 and fas-1A, resulting in synthesis of AFB1. An 8-kb SacI subclone of cosmid NorA complemented fas-1A only, resulting in accumulation of norsolorinic acid. Gene disruption of the fas-1A locus blocked norsolorinic acid accumulation in *A. parasiticus* B62 (nor-1), which normally accumulates this intermediate. These data confirmed that fas-1A is directly involved in AFB1 synthesis. The predicted amino acid sequence of fas-1A showed a high level of identity with extensive regions in the enoyl reductase and malonyl/palmitoyl transferase functional domains in the beta subunit of yeast fatty acid synthetase. Together, these data suggest that fas-1A encodes a novel fatty acid synthetase which synthesizes part of the polyketide backbone of AFB1. Additional data support an interaction between AFB1 synthesis and sclerotium development.

23/7/13 (Item 4 from file: 73)
DIALOG(R) File 73:EMBASE
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9773839 EMBASE No: 95325760

Purification of crotonyl-CoA reductase from *Streptomyces collinus* and cloning, sequencing and expression of the corresponding gene in *Escherichia coli*

Wallace K.K.; Bao Z.-Y.; Dai H.; Digate R.; Schuler G.; Speedie M.K.; Reynolds K.A.

School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD 21201 USA

European Journal of Biochemistry (Germany) , 1995, 233/3 (954-962)

CODEN: EJBCA ISSN: 0014-2956

LANGUAGES: English SUMMARY LANGUAGES: English

A crotonyl-CoA reductase (EC 1.3.1.38, acyl-CoA:NADP+ trans-2-oxidoreductase) catalyzing the conversion of crotonyl-CoA to butyryl-CoA has been purified and characterized from *Streptomyces collinus*. This enzyme, a dimer with subunits of identical mass (48 kDa), exhibits a $K_m = 18 \text{ microM}$ for crotonyl-CoA and 15 microM for NADPH. The enzyme was unable to catalyze the reduction of any other enoyl-CoA thioesters or to utilize NADH as an electron donor. A highly effective inhibition by straight-chain fatty acids ($K_i = 9.5 \text{ microM}$ for palmitoyl-CoA) compared with branched-chain fatty acids ($K_i > 400 \text{ microM}$ for isopalmitoyl-CoA) was observed. All of these properties are consistent with a proposed role of the enzyme in providing butyryl-CoA as a starter unit for straight-chain fatty acid biosynthesis. The crotonyl-CoA reductase gene was cloned in *Escherichia coli*. This gene, with a proposed designation of ccr, is encoded in a 1344-bp open reading frame which predicts a primary

translation product of 448 amino acids with a calculated molecular mass of 49.4 kDa. Several dispersed regions of highly significant sequence similarity were noted between the deduced amino acid sequence and various alcohol dehydrogenases and **fatty acid synthases**, including one region that contains a putative NADPH binding site. The ccr gene product was expressed in *E. coli* and the induced crotonyl-CoA reductase was purified tenfold and shown to have similar steady-state kinetics and electrophoretic mobility on sodium dodecyl sulfate/polyacrylamide to the native protein.

23/7/14 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE
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9288082 EMBASE No: 94245115

Isolation of cDNAs from *Brassica napus* encoding the biotin-binding and transcarboxylase domains of acetyl-CoA carboxylase: Assignment of the domain structure in a full-length *Arabidopsis thaliana* genomic clone

Elborough K.M.; Swinhoe R.; Winz R.; Kroon J.T.M.; Farnsworth L.; Fawcett T.; Martinez-Rivas J.M.; Slabas A.R.

Lipid Molecular Biology Group, Biological Sciences Department, University of Durham, South Road, Durham DH1 3LE United Kingdom

BIOCHEM. J. (United Kingdom), 1994, 301/2 (599-605) CODEN: BIJOA
ISSN: 0264-6021

LANGUAGES: English SUMMARY LANGUAGES: English

One independent and two overlapping rape cDNA clones have been isolated from a rape embryo library. We have shown that they encode a 2.3 kb and a 2.5 kb stretch of the full-length acetyl-CoA carboxylase (ACCase) cDNA, corresponding to the biotin-binding and transcarboxylase domains respectively. Using the cDNA in Northern-blot analysis we have shown that the mRNA for ACCase has a higher level of expression in rape seed than in rape leaf and has a full length of 7.5 kb. The level of expression during rape embryogenesis was compared with both oil deposition and expression of two fatty acid synthetase components enoyl-(acyl-carrier-protein) reductase and 3-oxoacyl-(acyl-carrier-protein) reductase. Levels of ACCase mRNA were shown to peak at 29 days after anthesis during embryonic development, similarly to enoyl-(acyl-carrier-protein) reductase and 3-oxoacyl-(acyl-carrier-protein) reductase mRNA. In addition, a full-length genomic clone (19 kb) of *Arabidopsis* ACCase has been isolated and partially sequenced. Analysis of the clone has allowed the first **plant** ACCase activity domains (biotin carboxylase-biotin binding-transcarboxylase) to be ordered and assigned. Southern blot analysis using the *Arabidopsis* clone indicates that ACCase is a single-copy gene in *Arabidopsis* but is encoded by a small gene family in rape.

23/7/15 (Item 6 from file: 73)
DIALOG(R)File 73:EMBASE
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8903352 EMBASE No: 93207108

Multiple inhibitory effects of garlic extracts on cholesterol biosynthesis in hepatocytes

Gebhardt R.

Physiologisch-Chemisches Institut, University of Tübingen, Hoppe-Seyler-Str. 4, D-72076 Tübingen Germany

LIPIDS (USA), 1993, 28/7 (613-619) CODEN: LPDSA ISSN: 0024-4201

LANGUAGES: English SUMMARY LANGUAGES: English

Exposure of primary rat hepatocytes and human HepG2 cells to water-soluble garlic extracts resulted in the concentration-dependent inhibition of cholesterol biosynthesis at several different enzymatic steps. At low concentrations, sterol biosynthesis from (14C)acetate was decreased in rat hepatocytes by 23% with an IC50 (half-maximal inhibition) value of 90 microg/mL and in HepG2 cells by 28% with an IC50 value of 35 microg/mL.

This inhibition was exerted at the level of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) as indicated by direct enzymatic measurements and the absence of inhibition if (14C)mevalonate was used as a precursor. At high concentrations (above 0.5 mg/mL), inhibition of cholesterol biosynthesis was not only seen at an early step where it increased considerably with dose, but also at later steps resulting in the accumulation of the precursors lanosterol and 7-dehydrocholesterol. No desmosterol was formed which, however, was a major precursor accumulating in the presence of triparanol. Thus, the accumulation of sterol precursors seems to be of less therapeutic significance during consumption of garlic, because it requires concentrations one or two orders of magnitude above those affecting HMG-CoA reductase. Alliin, the main sulfur-containing compound of garlic, was without effect itself. If converted to allicin, it resulted in similar changes of the sterol pattern. This suggested that the latter compound might contribute to the inhibition at the late steps. In contrast, nicotinic acid and particularly adenosine caused moderate inhibition of HMG-CoA reductase activity and of cholesterol biosynthesis suggesting that these compounds participate, at least in part, in the early inhibition of sterol synthesis by garlic extracts.

23/7/16 (Item 7 from file: 73)
DIALOG(R)File 73:EMBASE
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8630876 EMBASE No: 92306786

Molecular cloning and sequencing of the gene for mycocerosic acid **synthase**, a novel **fatty acid** elongating multifunctional enzyme, from *Mycobacterium tuberculosis* var. *bovis* Bacillus Calmette-Guerin Mathur M.; Kolattukudy P.E.

Ohio State Biotechnology Center, 206 Rightmire Hall, Ohio State University, 1060 Carmack Rd., Columbus, OH 43210 USA

J. BIOL. CHEM. (USA) , 1992, 267/27 (19388-19395) CODEN: JBCHA ISSN: 0021-9258

LANGUAGES: English SUMMARY LANGUAGES: English

Mycocerosyl lipids are found uniquely in the cell walls of pathogenic mycobacteria. Mycocerosic acid synthase (MAS) is a multifunctional protein which catalyzes elongation of n-fatty acyl-CoA with methylmalonyl-CoA as the elongating agent (Rainwater, D. L., and Kolattukudy, P. E. (1985) J. Biol. Chem. 260, 616-623). To understand how the various domains that catalyze the reactions involved in chain elongation are organized, mas gene from *Mycobacterium tuberculosis bovis* BCG was cloned. A lambda gt11 library of AluI partially digested genomic DNA from the organism was screened with an oligonucleotide probe designed from the N-terminal amino acid sequence of purified MAS. Using terminal segments of inserts from positive clones as the probe, the library was rescreened and the process was repeated. Sequencing of four overlapping clones revealed a contiguous sequence of 9699 base pair(s) (bp) of mycobacterial genome containing a 6330-bp open reading frame that could code for a protein of 2100 amino acids with a molecular mass of 225,437 daltons. The authenticity of the open reading frame as that of MAS was verified by correspondence of the amino acid sequences deduced from the gene with the directly determined amino acid sequences of the N terminus and three different internal peptide fragments. By comparing the MAS amino acid sequence with the sequences in the active site regions of known **fatty acid synthases** and polyketide **synthases** the functional domains in MAS were identified. This analysis showed that the domains were organized in the following order: beta-ketoacyl synthase, acyl transferase, dehydratase-enoyl reductase, beta-ketoreductase, acyl carrier protein; no thioesterase-like domain could be found. These results establish MAS as the first case of an elongating multifunctional enzyme composed of two identical subunits that resemble the vertebrate **fatty acid synthase** in size, subunit structure, and linear organization of functional domains. Southern and Western blot analyses showed absence of mas gene and encoded proteins in *Mycobacterium smegmatis* and *Escherichia coli*. This result is consistent

with the report that mycocerosic acid is present only in pathogenic mycobacteria.

23/7/17 (Item 8 from file: 73)

DIALOG(R)File 73:EMBASE

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8506188 EMBASE No: 92182162

Isolation and characterization of a cDNA from *Cuphea lanceolata* encoding a beta-ketoacyl-ACP reductase

Klein B.; Pawlowski K.; Horicke-Grandpierre C.; Schell J.; Topfer R.

Max-Planck-Inst. fur Zuchtungsforsch, Carl-von-Linne Weg 10, W-5000 Koln 30 Germany

MOL. GEN. GENET. (Germany) , 1992, 233/1-2 (122-128) CODEN: MGGEA

ISSN: 0026-8925 ADONIS ORDER NUMBER: 002689259200144F

LANGUAGES: English SUMMARY LANGUAGES: English

A cDNA encoding beta-ketoacyl-ACP reductase (EC 1.1.1.100), an integral part of the **fatty acid synthase** type II, was cloned from *Cuphea lanceolata*. This cDNA of 1276 bp codes for a polypeptide of 320 amino acids with 63 N-terminal residues presumably representing a transit peptide and 257 residues corresponding to the mature protein of 27 kDa. The encoded protein shows strong homology with the amino-terminal sequence and two tryptic peptides from avocado mesocarp beta-ketoacyl-ACP reductase, and its total amino acid composition is highly similar to those of the beta-ketoacyl-ACP reductases of avocado and spinach. Amino acid sequence homologies to polyketide synthase, beta-ketoreductases and short-chain alcohol dehydrogenases are discussed. An engineered fusion protein lacking most of the transit peptide, which was produced in *Escherichia coli*, was isolated and proved to possess beta-ketoacyl-ACP reductase activity. Hybridization studies revealed that in *C. lanceolata* beta-ketoacyl-ACP reductase is encoded by a small family of at least two genes and that members of this family are expressed in roots, leaves, flowers and seeds.

23/7/18 (Item 9 from file: 73)

DIALOG(R)File 73:EMBASE

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8139552 EMBASE No: 91169325

The pentafunctional FAS1 genes of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are co-linear and considerably longer than previously estimated

Kottig H.; Rottner G.; Beck K.-F.; Schweizer M.; Schweizer E.

Lehrstuhl fur Biochemie, Universitat Erlangen-Nurnberg, W-8520 Erlangen Germany, Federal Republic of

MOL. GEN. GENET. (Germany, Federal Republic of) , 1991, 226/1-2 (310-314)

CODEN: MGGEA ISSN: 0026-8925 ADONIS ORDER NUMBER: 002689259100129X

LANGUAGES: English

The fatty acid synthetase (FAS) gene FAS1 of the alkane-utilizing yeast *Yarrowia lipolytica* was cloned and sequenced. The gene is represented by an intron-free reading frame of 6228 bp encoding a protein of 2076 amino acids and 229980 Da molecular weight. This protein exhibits a 58% sequence similarity to the corresponding *Saccharomyces cerevisiae* FAS beta-subunit. The sequential order of the five FAS1-encoded enzyme domains, acetyl transferase, enoyl reductase, dehydratase and malonyl/palmitoyl-transferase, is co-linear in both organisms. This finding agrees with available evidence that the functional organization of FAS genes is similar in related organisms but differs considerably between unrelated species. In addition, previously reported conflicting data concerning the 3' end of *S. cerevisiae* FAS1 were re-examined by genomic and cDNA sequencing of the relevant portion of the gene. Thereby, the translational stop codon was shown to lie considerably downstream of both published termination sites. The *S. cerevisiae* FAS1 gene thus has a corrected length of 6153 bp and encodes a protein of 2051 amino acids and 228667 Da molecular weight.

23/7/19 (Item 10 from file: 73)
DIALOG(R)File 73:EMBASE
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6033754 EMBASE No: 86028814

Suppression of cholesterologenesis by **plant** constituents. Review of Wisconsin contributions to NC-167

Qureshi A.A.; Burger W.C.; Peterson D.M.; Elson C.
Cereal Crops Research Unit, Madison, Wi 53705 USA
LIPIDS (USA) , 1985, 20/11 (817-824) CODEN: LPDSA
LANGUAGES: ENGLISH

In animals, non-sterol metabolites of the mevalonate pathway act independently from receptor-mediated cholesterol uptake in the multivalent feedback regulation of mevalonate biosynthesis. Studies leading to the isolation and characterization of **plant** -borne suppressors of mevalonate biosynthesis are reviewed. We propose that one cardio-protective component of the vegetarian diet consists of a variety of non-sterol, post-mevalonate metabolites. These products of **plant** branches of the mevalonate pathway, discarded as animals evolved, continue to influence animal sterol metabolism. It is through this action, we propose, that the cholesterol-suppressive action of **plant** materials is expressed.

23/7/20 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07503150 93192253

Low carbon monoxide affinity allene oxide synthase is the predominant cytochrome P450 in many **plant** tissues.

Lau SM; Harder PA; O'Keefe DP
Central Research and Development, Dupont Company, Experimental Station, Wilmington, Delaware 19880-0402.

Biochemistry (UNITED STATES) Mar 2 1993, 32 (8) p1945-50, ISSN 0006-2960 Journal Code: AOG

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A cytochrome P450 with low affinity (2.9×10^3 M⁻¹) for CO appears to be the major microsomal P450 in some **plant** tissues. The presence of low CO affinity cytochrome P450 correlates with its lack of NADPH reducibility and with the presence of high levels of 13(S)-hydroperoxy-9(Z),11(E)-octadecadienoate peroxidase activity. This activity and low CO affinity are retained by purified tulip cytochrome P450, which appears to be catalytically identical to a flaxseed-derived **fatty acid** allene oxide **synthase** P450 described previously [Song, W.-C., & Brash, A.R. (1991) Science 253, 781-784]. Other heme-binding ligands, such as CN- and imidazoles, bind weakly to the allene oxide synthase P450s, suggesting that axial coordination in the heme distal pocket may be hindered. We conclude that low CO affinity is characteristic of the allene oxide synthase P450s and that these P450s constitute a major portion of the microsomal P450 in a variety of **plant** tissues, particularly from monocot species.

23/7/21 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1997 Knight-Ridder Info. All rts. reserv.

06832411 92003699

cdNA cloning and expression of Brassica napus enoyl-acyl carrier protein reductase in Escherichia coli.

Kater MM; Koningstein GM; Nijkamp HJ; Stuitje AR
Department of Genetics, Vrije Universiteit, Amsterdam, Netherlands.

Plant Mol Biol (NETHERLANDS) Oct 1991, 17 (4) p895-909, ISSN

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The onset of storage lipid biosynthesis during seed development in the oilseed crop *Brassica napus* (rape seed) coincides with a drastic qualitative and quantitative change in fatty acid composition. During this phase of storage lipid biosynthesis, the enzyme activities of the individual components of the **fatty acid synthase** system increase rapidly. We describe a rapid and simple purification procedure for the plastid-localized NADH-dependent enoyl-acyl carrier protein reductase from developing *B. napus* seed, based on its affinity towards the acyl carrier protein (ACP). The purified protein was N-terminally sequenced and used to raise a potent antibody preparation. Immuno-screening of a seed-specific lambda gt11 cDNA expression library resulted in the isolation of enoyl-ACP reductase cDNA clones. DNA sequence analysis of an apparently full-length cDNA clone revealed that the enoyl-ACP reductase mRNA is translated into a precursor protein with a putative 73 amino acid leader sequence which is removed during the translocation of the protein through the plastid membrane. Expression studies in *Escherichia coli* demonstrated that the full-length cDNA clone encodes the authentic *B. napus* NADH-dependent enoyl-ACP reductase. Characterization of the enoyl-ACP reductase genes by Southern blotting shows that the allo-tetraploid *B. napus* contains two pairs of related enoyl-ACP reductase genes derived from the two distinct genes found in both its ancestors, *Brassica oleracea* and *B. campestris*. Northern blot analysis of enoyl-ACP reductase mRNA steady-state levels during seed development suggests that the increase in enzyme activity during the phase of storage lipid accumulation is regulated at the level of gene expression.

23/7/22 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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06461263 90234108

Stilbene and chalcone synthases: related enzymes with key functions in **plant**-specific pathways.

Schroder J; Schroder G

Institute fur Biologie II, Universitat Freiburg, Bundesrepublik Deutschland.

Z Naturforsch [C] (GERMANY, WEST) Jan-Feb 1990, 45 (1-2) p1-8, ISSN 0341-0382 Journal Code: ACL

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW LITERATURE

Several years of extensive research using the new powerful techniques of molecular biology have enabled the direct comparison of functionally or evolutionarily related genes and their products at the nucleotide and amino acid sequence levels. Two types of synthase with similar functions are discussed as an interesting example. Stilbene synthases, e.g. resveratrol synthase, produce the stilbene backbone as a key reaction in the biosynthesis of stilbene-type phytoalexins. Chalcone synthase is a key enzyme in the biosynthesis of flavonoids, including certain phytoalexins derived from a 6'-deoxychalcone which is synthesized by cooperation of chalcone synthase with a reductase. Resveratrol and chalcone synthases utilize the same substrates (4-coumaroyl-CoA and 3 molecules of malonyl-CoA) and catalyze the same condensing type of enzyme reaction (resulting in sequential addition of acetate units via malonyl-CoA), but the products differ in the newly formed ring systems (resveratrol and naringenin chalcone). A comparative analysis of cloned DNA sequences and of the reaction mechanisms indicates that the two enzymes are closely related. It seems likely that the proteins possess a common scaffold for substrate recognition and for the condensing reaction, and that the different folding of an enzyme-bound intermediate prior to closure of the new aromatic ring is responsible for the formation of the different products. The same type of condensing reaction is utilized by the 2-ketoacyl-ACP **synthases** of

fatty-acid biosynthesis. However, the available data indicate that these enzymes share little overall homology with either resveratrol or chalcone synthase. One exception may be a short amino acid sequence which corresponds to the active center of the condensing reaction in 2-ketoacyl-ACP synthases. (41 Refs.)

23/7/23 (Item 4 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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05566508 88240437

Regulation of hepatic cholesterol biosynthesis by fatty acids: effect of feeding olive oil on cytoplasmic acetoacetyl-coenzyme A thiolase, beta-hydroxy-beta-methylglutaryl-CoA synthase, and acetoacetyl-coenzyme A ligase.

Salam WH; Cagen LM; Heimberg M
Department of Pharmacology, College of Medicine, University of Tennessee, Memphis 38163.

Biochem Biophys Res Commun (UNITED STATES) May 31 1988, 153 (1) p422-7
, ISSN 0006-291X Journal Code: 9Y8

Contract/Grant No.: HL-27850, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We reported previously that, in the perfused rat liver, oleic acid increased the specific activity of cytosolic enzymes of cholesterol biosynthesis. In this study, we examined the effects of oral administration of olive oil on the activities of HMG-CoA synthase, AcAc-CoA thiolase, AcAc-CoA ligase and HMG-CoA reductase. Olive oil feeding increased the specific activity of hepatic HMG-CoA synthase by 50%, AcAc-CoA thiolase by 2-fold, and AcAc-CoA ligase by 3-fold. Olive oil had no effect on HMG-CoA reductase activity. These data suggest that the enzymes that supply the HMG-CoA required for hepatic cholesterologenesis are regulated in parallel by a physiological substrate, **fatty acid**, independent of HMG-CoA **reductase** under these conditions.

23/7/24 (Item 1 from file: 351)
DIALOG(R) File 351:DERWENT WPI
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010409548

WPI Acc No: 95-310894/199540

DNA construct expressing jojoba wax synthase and transformed Brassica cells - useful for producing wax ester(s) for use in pharmaceuticals and cosmetics, etc

Patent Assignee: CALGENE INC (CALJ)

Inventor: LARDIZABAL K D; LASSNER M W; METZ J G

Number of Countries: 001 Number of Patents: 001

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
US 5445947	A	19950829	US 91796256	A	19911120	C12P-001/04	199540 B
			US 92933411	A	19920821		
			WO 92US9863	A	19921113		
			US 9366299	A	19930520		

Priority Applications (No Type Date): US 9366299 A 19930520; US 91796256 A 19911120; US 92933411 A 19920821; WO 92US9863 A 19921113

Patent Details:

Patent	Kind	Lan	Pg	Filing Notes	Application	Patent
US 5445947	A		50	CIP of	US 91796256	
				CIP of	US 92933411	
				CIP of	WO 92US9863	

Abstract (Basic): US 5445947 A

A recombinant DNA construct is new which comprises a nucleic acid sequence (I) encoding the 524 or 521 amino acid proteins and a heterologous DNA sequence (II) not naturally associated with (I). Also new is a Brassica **plant** cell which contains a construct as above which encodes a protein that is heterologous to the host, under control of a promoter functional in the host cell.

USE - (I) encodes fatty acyl-CoA: fatty alcohol O-acyltransferase ('wax synthase') from jojoba (*Simmondsia chinensis*). This enzyme is involved in biosynthesis of wax esters from fatty alcohols and fatty acyl substrates. (I) is used for prodn. of recombinant wax synthase or to isolate related sequences from other organisms, while the enzyme is used to produce wax esters in cells that do not normally produce it (partic. when the cells are also engineered to express a **fatty acyl reductase**). Wax esters are useful in pharmaceuticals, cosmetics, detergents, plastics and lubricants.

Dwg.0/3

Derwent Class: B04; D16; D21; P14

International Patent Class (Main): C12P-001/04

International Patent Class (Additional): A01M-001/00; C12N-015/05;

C12P-007/64

23/7/25 (Item 2 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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009887559

WPI Acc No: 94-167474/199420

Recombinant DNA encoding gene inhibitor proteins, expressed in initial feeding cell or nematode feeding structure - useful for producing transgenic **plants** having reduced susceptibility to **plant** parasitic nematodes

Patent Assignee: MOGEN INT NV (MOGE-N)

Inventor: GODDIJN O J M; SIJMONS P C; VAN DEN ELZEN P J M; VAN DER LEE F M

Number of Countries: 045 Number of Patents: 003

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
WO 9410320	A1	19940511	WO 93EP3091	A	19931102	C12N-015/82	199420 B
AU 9454205	A	19940524	WO 93EP3091	A	19931102	C12N-015/82	199434
			AU 9454205	A	19931102		
EP 666922	A1	19950816	EP 93924590	A	19931102	C12N-015/82	199537
			WO 93EP3091	A	19931102		

Priority Applications (No Type Date): EP 92203378 A 19921102

Cited Patents: 05 journal ref.; EP 458367; EP 480730; EP 502730; WO 9002172
; WO 9204453; WO 9221757

Patent Details:

Patent	Kind	Lan	Pg	Filing	Notes	Application	Patent
WO 9410320	A1	E	43				
Designated States (National): AU BB BG BR BY CA CZ FI HU JP KP KR KZ LK MG MN MW NO NZ PL RO RU SD SK UA US VN							
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE							
AU 9454205	A			Based on		WO 9410320	
EP 666922	A1	E		Based on		WO 9410320	
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE							

Abstract (Basic): WO 9410320 A

Recombinant DNA contg. a **plant** expressible gene comprises in sequence: (a) a promoter capable of driving the expression of a downstream gene in an initial feeding cell and/or a nematode feeding structure, (b) a gene encoding a prod. that inhibits an endogenous gene encoding ATP synthetase, adenine nucleotide translocator, di- or tricarboxylate translocator, 2-oxo-glutarate translocator, cytochrome

C, pyruvate kinase, glyceraldehyde-3P- dehydrogenase, NADPH-cytochrome p450 **reductase**, **fatty acid synthase** complex, glycerol-3P-acetyltransferase, hydroxymethyl-glutaryl CoA reductase, aminoacyl transferase, or a transcription initiation or elongation factor, and (c) opt. a transcription terminator and a polyadenylation signal sequence where the gene is expressed upon infection by the nematode.

The recombinant DNA comprises an RNA transcript that is at least partially complementary to the endogenous gene transcript. The promoter is from the Delta-0.3TobRB7-5A promoter. The replicon is a Ti- or Ri-plasmid from Agrobacterium. It is capable of replication in E. coli and Agrobacterium. The **plant** is a member of the family Solanum, esp. S. tuberosum. The **plant** parasitic nematode is a Meloidogyne sp.

USE - **Plants** transformed with the recombinant DNA have reduced susceptibility to **plant** parasitic nematodes. Reduced crop damage from nematodes can be achieved using the DNA.

Dwg.0/5

Derwent Class: C06; D16; P13

International Patent Class (Main): C12N-015/82

International Patent Class (Additional): A01H-005/00

23/7/26 (Item 1 from file: 10)

DIALOG(R)File 10:AGRICOLA

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3560994 20553595 Holding Library: AGL

Isolation and characterization of peroxisomes from diatoms

Winkler, U. Stabenau, H.

Universitat Oldenburg, Oldenburg, Germany.

Berlin ; New York : Springer-Verlag, 1925-

Planta. 1995. v. 195 (3) p. 403-407.

ISSN: 0032-0935 CODEN: PLANAB

DNAL CALL NO: 450 P693

Language: English

Includes references

Place of Publication: Germany, West

Subfile: IND; OTHER FOREIGN;

Document Type: Article

Peroxisomes were isolated by gradient centrifugation from two different diatoms: *Nitzschia laevis* (subgroup of Pennales) and *Thalassiosira fluviatilis* (subgroup of Centrales). In neither of these organelles could catalase or any H₂O₂-forming oxidase be demonstrated. The glycolate-oxidizing enzyme present in the peroxisomes is a dehydrogenase capable of oxidizing L-lactate as well. The peroxisomes also contain the glyoxysomal markers isocitrate lyase and malate **synthase**. However, enzymes of the **fatty-acid** beta-oxidation pathway are located exclusively in the mitochondria. The mitochondria additionally possess glutamate-glyoxylate aminotransferase and a glycolate dehydrogenase which differs from the peroxisomal glycolate dehydrogenase since it preferably utilizes D-lactate as an alternative substrate. Hydroxypyruvate reductase and glyoxylate carboligase were not found in the cells of either diatom. By culturing *Nitzschia laevis* it could be demonstrated that decreasing the CO₂ concentration in the aeration mixture from 2% to 0.03% and increasing the irradiance from 40 to 250 micromole quanta m⁻¹ s⁻¹ resulted in an increase of all peroxisomal enzyme activities. In addition, enzyme activities of the beta-oxidation pathway were increased. However, mitochondrial glycolate dehydrogenase and amino transferase did not alter their activities under these conditions. Summarizing all results, it is postulated that there are two different pathways for the metabolism of glycolate in the diatoms.

23/7/27 (Item 2 from file: 10)

DIALOG(R)File 10:AGRICOLA

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3419964 20439710 Holding Library: AGL

Developmental specific expression and organelle targeting of the *Escherichia coli* fabD gene, encoding malonyl coenzyme A-acyl carrier protein transacylase in transgenic rape and tobacco seeds

Verwoert, I.I.G.S. Linden, K.H. van der.; Nijkamp, H.J.J.; Stuitje, A.R.

Vrije Universiteit, Amsterdam, Netherlands.

Dordrecht : Kluwer Academic Publishers.

Plant molecular biology. Oct 1994. v. 26 (1) p. 189-202.

ISSN: 0167-4412 CODEN: PMBIDB

DNAL CALL NO: QK710.P62

Language: English

Includes references

Place of Publication: Netherlands

Subfile: IND; OTHER FOREIGN;

Document Type: Article

In both **plants** and bacteria, de novo fatty acid biosynthesis is catalysed by a type II fatty acid synthetase (FAS) system which consists of a group of eight discrete enzyme components. The introduction of heterologous, i.e. bacterial, FAS genes in **plants** could provide an alternative way of modifying the **plant** lipid composition. In this study the *Escherichia coli* fabD gene, encoding malonyl CoA-ACP transacylase (MCAT), was used as a model gene to investigate the effects of over-producing a bacterial FAS component in the seeds of transgenic **plants**. Chimeric genes were designed, so as not to interfere with the household activities of fatty acid biosynthesis in the earlier stages of seed development, and introduced into tobacco and rapeseed using the *Agrobacterium tumefaciens* binary vector system. A napin promoter was used to express the *E. coli* MCAT in a seed-specific and developmentally specific manner. The rapeseed enoyl-ACP reductase transit peptide was used successfully, as confirmed by immunogold labelling studies, for plastid targeting of the bacterial protein. The activity of the bacterial enzyme reached its maximum (up to 55 times the maximum endogenous MCAT activity) at the end of seed development, and remained stable in mature transgenic seeds. Significant changes in fatty acid profiles of storage lipids and total seed lipid content of the transgenic **plants** were not found. These results are in support of the notion that MCAT does not catalyse a rate-limiting step in **plant** fatty acid biosynthesis.

23/7/28 (Item 3 from file: 10)

DIALOG(R)File 10:AGRICOLA

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3407406 20428848 Holding Library: AGL

The use of a hybrid genetic system to study the functional relationship between prokaryotic and **plant** multi-enzyme fatty acid synthetase

Kater, M.M. Koningstein, G.M.; Nijkamp, H.J.J.; Stuitje, A.R.

Dordrecht : Kluwer Academic Publishers.

Plant molecular biology. Aug 1994. v. 25 (5) p. 771-790.

ISSN: 0167-4412 CODEN: PMBIDB

DNAL CALL NO: QK710.P62

Language: English

Includes references

Place of Publication: Netherlands

Subfile: IND; OTHER FOREIGN;

Document Type: Article

Fatty acid synthesis in bacteria and **plants** is catalysed by a multi-enzyme fatty acid synthetase complex (FAS II) which consists of separate monofunctional polypeptides. Here we present a comparative molecular genetic and biochemical study of the enoyl-ACP reductase FAS components of **plant** and bacterial origin. The putative bacterial enoyl-ACP reductase gene (envM) was identified on the basis of amino acid

sequence similarities with the recently cloned **plant** enoyl-ACP reductase. Subsequently, it was unambiguously demonstrated by overexpression studies that the envM gene encodes the bacterial enoyl-ACP reductase. An anti-bacterial agent called diazaborine was shown to be a specific inhibitor of the bacterial enoyl-ACP reductase, whereas the **plant** enzyme was insensitive to this synthetic antibiotic. The close functional relationship between the **plant** and bacterial enoyl-ACP reductases was inferred from genetic complementation of an envM mutant of Escherichia coli. Ultimately, envM gene-replacement studies, facilitated by the use of diazaborine, demonstrated for the first time that a single component of the **plant** FAS system can functionally replace its counterpart within the bacterial multi-enzyme complex. Finally, lipid analysis of recombinant E. coli strains with the hybrid FAS system unexpectedly revealed that enoyl-ACP reductase catalyses a rate-limiting step in the elongation of unsaturated fatty acids.

23/7/29 (Item 4 from file: 10)
DIALOG(R)File 10:AGRICOLA
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3213856 92057489 Holding Library: AGL
Diflufenican, a carotenogenesis inhibitor, also reduces acyl lipid synthesis

Ashton, I.P. Abulnaja, K.O.; Pallett, K.E.; Cole, D.J.; Harwood, J.L.
University of Wales College of Cardiff, Cardiff, UK
Orlando, Fla. : Academic Press.
Pesticide biochemistry and physiology. May 1992. v. 43 (1) p. 14-21.
ISSN: 0048-3575 CODEN: PCBPB
DNAL CALL NO: SB951.P49
Language: English
Includes references.
Subfile: OTHER US (NOT EXP STN, EXT, USDA; SINCE 12/76);
Document Type: Article

The bleaching herbicide diflufenican (DFF) has been shown to produce effects on the membranes of sensitive **plant** tissues which may be independent of its inhibition of carotenoid synthesis. Therefore, we examined whether DFF had any action on acyl lipid metabolism. In leaves from a variety of **plants**, DFF was shown to inhibit the incorporation of radioactivity from [1-14C]acetate and [2-14C]malonate into acyl lipids. The labeling of all lipid classes was reduced equally and there was no change in the pattern of fatty acids labeled, suggesting that fatty acid synthesis de novo was affected. Further experiments suggested that fatty acid synthetase rather than acetyl-CoA carboxylase was the site of action. Direct measurement of fatty acid synthetase in soluble fractions from pea and barley leaves and avocado mesocarp confirmed that DFF inhibited the enzyme complex in vitro. The inhibition was competitive against pyridine nucleotides and suggested that one or both of the **reductase** components of **fatty acid** synthetase was the target site.

23/7/30 (Item 5 from file: 10)
DIALOG(R)File 10:AGRICOLA
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3100686 91034136 Holding Library: AGL
The pentafunctional FAS1 genes of Saccharomyces cerevisiae and Yarrowia lipolytica are co-linear and considerably longer than previously estimated
Kotting, H. Rottner, G.; Beck, K.F.; Schweizer, M.; Schweizer, E.
Universitat Erlangen-Nurnberg, Erlangen, FRG
Berlin, W. Ger. : Springer International.
M G G : Molecular and general genetics. Apr 1991. v. 226 (1/2) p. 310-314.
ISSN: 0026-8925 CODEN: MGGEAE
DNAL CALL NO: 442.8 Z34

Language: English
Includes references.
Subfile: OTHER FOREIGN;
Document Type: Article

The fatty acid synthetase (FAS) gene FAS1 of the alkane-utilizing yeast *Yarrowia lipolytica* was cloned and sequenced. The gene is represented by an intron-free reading frame of 6228 bp encoding a protein of 2076 amino acids and 229,980 Da molecular weight. This protein exhibits a 58% sequence similarity to the corresponding *Saccharomyces cerevisiae* FAS beta-subunit. The sequential order of the five FAS1-encoded enzyme domains, acetyl transferase, enoyl reductase, dehydratase and malonyl/palmitoyl-transferase, is co-linear in both organisms. This finding agrees with available evidence that the functional organization of FAS genes is similar in related organisms but differs considerably between unrelated species. In addition, previously reported conflicting data concerning the 3' end of *S. cerevisiae* FAS1 were re-examined by genomic and cDNA sequencing of the relevant portion of the gene. Thereby, the translational stop codon was shown to lie considerably downstream of both published termination sites. The *S. cerevisiae* FAS1 gene thus has a corrected length of 6153 bp and encodes a protein of 2051 amino acids and 228 667 Da molecular weight.

? b 5,35,73,155,351,10,203

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\$0.00 Estimated cost this search
\$0.00 Estimated total session cost 0.119 Hrs.

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File 5:BIOSIS PREVIEWS(R) 1969-1997/Sep W4
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Processing

111232	SYNTHASE?
1176881	GENE
563010	GENES
358223	FATTY
44885	WAX
2052788	PLANT
859160	PLANTS
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Processing

Processing

S211077447 PY>1991
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S3 29	S1 NOT S2

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4/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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8202420 BIOSIS Number: 91123420

STEAROYLACYL-CARRIER-PROTEIN DESATURASE FROM HIGHER **PLANTS** IS
STRUCTURALLY UNRELATED TO THE ANIMAL AND FUNGAL HOMOLOGS

SHANKLIN J; SOMERVILLE C

DEP. ENERGY PLANT RES. LAB., MICHIGAN STATE UNIV., EAST LANSING, MICH.
48824.

PROC NATL ACAD SCI U S A 88 (6). 1991. 2510-2514. CODEN: PNASA

Full Journal Title: Proceedings of the National Academy of Sciences of
the United States of America

Language: ENGLISH

Stearoyl-acyl-carrier-protein (ACP) desaturase (EC 1.14.99.6) was purified to homogeneity from avocado mesocarp, and monospecific polyclonal antibodies directed against the protein were used to isolate full-length cDNA clones from *Ricinus communis* (castor) seed and *Cucumis sativus* (cucumber). The nucleotide sequence of the castor clone pRCD1 revealed an open reading frame of 1.2 kilobases encoding a 396-amino acid protein of 45 kDa. The cucumber clone pCSD1 encoded a homologous 396-amino acid protein with 88% amino acid identity to the castor clone. Expression of pRCD1 in *Saccharomyces cerevisiae* resulted in the accumulation of a functional stearoyl-ACP desaturase, demonstrating that the introduction of this single **gene** product was sufficient to confer soluble desaturase activity to yeast. There was no detectable identity between the deduced amino acid sequences of the castor .DELTA.9-stearoyl-ACP desaturase and either the .DELTA.9-stearoyl-CoA desaturase from rat or yeast or the .DELTA.12 desaturase from *Synechocystis*, suggesting that these enzymes may have evolved independently. However, there was a 48-residue region of 29% amino acid-sequence identity between residues 53 and 101 of the castor desaturase and the proximal border of the dehydratase region of the **fatty acid synthase** from yeast. Stearoyl-ACP mRNA was present at substantially higher levels in developing seeds than in leaf and root tissue, suggesting that expression of the .DELTA.9 desaturase is developmentally regulated.

4/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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7771229 BIOSIS Number: 90139229

THE MULTIFUNCTIONAL 6 METHYLSALICYLIC ACID **SYNTHASE GENE** OF
PENICILLIUM-PATULUM ITS **GENE** STRUCTURE RELATIVE TO THAT OF OTHER
POLYKETIDE **SYNTHASES**

BECK J; RIPKA S; SIEGNER A; SCHILTZ E; SCHWEIZER E

LEHRSTUHL FUER BIOCHEMIE DER UNIVERSITAET ERLANGEN-NUERNBERG,
STAUDTSTRASSE 5, D-8520 ERLANGEN, FRG.

EUR J BIOCHEM 192 (2). 1990. 487-498. CODEN: EJBCA

Full Journal Title: European Journal of Biochemistry

Language: ENGLISH

6-Methylsalicylic acid **synthase** (MSAS) from *Penicillium patulum* is a homomultimer of a single, multifunctional protein subunit. The enzyme is induced, at the transcriptional level, during the end of the logarithmic growth phase. After approximately 150-fold purification, a homogeneous enzyme preparation was obtained exhibiting, upon SDS gel electrophoresis, a subunit molecular mass of 188 kDa. By immunological screening of a genomic *P. patulum* DNA expression library, the MSAS **gene** together with its flanking sequences was isolated; 7131 base pairs of the cloned genomic DNA were sequenced. Within this sequence the MSAS **gene** was identified as a 5322-bp-long open reading frame coding for a protein of 1774 amino acids and 190731 Da molecular mass. Transcriptional initiation and termination

sites were determined both by primer extension studies and from cDNA sequences specially prepared for the 5' and 3' portions of the **gene**. The same cDNA sequences revealed the presence of a 69-bp intron within the N-terminal part of the MSAS **gene**. The intron contains the canonical GT and AG dinucleotides at its 5'- and 3'-splice junctions. An internal TACTGAC sequence, resembling the TACTAAC consensus element of *Saccharomyces cerevisiae* introns is suggested to represent the branch point of the lariat splicing intermediate. When compared to other known polyketide **synthases**, distinct amino acid sequence similarities of limited lengths were observed with some, though not all, of them. A comparatively low degree of similarity was detected to the yeast and *Penicillium* FAS or to the **plant** chalcone and resveratrol **synthases**. In contrast, a significantly higher sequence similarity was found between MSAS and the rat **fatty acid synthase**, especially at their transacyl reductase, 2-oxoacylase, 2-oxoacyl **synthase** and acyl carrier protein domains. Besides several dissimilar, interspersed regions probably coding for MSAS- and FAS-specific functions, the sequential order of the similar domains was colinear in both enzymes. The low similarity between the two *P. patulum* polyketide **synthases**, MSAS and FAS, possibly supports a convergent rather than a divergent evolution of both multienzyme proteins.

4/7/3 (Item 3 from file: 5)
 DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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7184405 BIOSIS Number: 88107150
 ANALYSIS OF THE NUCLEOTIDE SEQUENCE OF THE STREPTOMYCES-GLAUDESCENS TCML
GENES PROVIDES KEY INFORMATION ABOUT THE ENZYMOLOGY OF POLYKETIDE
 ANTIBIOTIC BIOSYNTHESIS
 BIBB M J; BIRO S; MOTAMEDI H; COLLINS J F; HUTCHINSON C R
 JOHN INNES INST., COLNEY LANE, NORWICH NR4 7UH, UK.
 EMBO (EUR MOL BIOL ORGAN) J 8 (9). 1989. 2727-2736. CODEN: EMJOD
 Full Journal Title: EMBO (European Molecular Biology Organization)
 Journal

Language: ENGLISH
 Key information about the biosynthesis of polyketide metabolites has been uncovered by sequence analysis of the tetracenomycin C polyketide **synthase genes** (tcmI) from *Streptomyces glaucescens* GLA.O. The sequence data revealed the presence of three complete open reading frames (ORFs). ORF1 and ORF2 appear to be translationally coupled and would encode proteins containing 426 and 405 amino acids, respectively. The two deduced proteins are homologous to known .beta.-ketoacyl **synthases**. ORF3 begins 70 nucleotides after the stop codon of ORF2 and would code for an 83 amino acid protein with a strong resemblance to known bacterial, animal and **plant** acyl-carrier proteins (ACP). The presence of an ACP **gene** within the tcm **gene** cluster suggests that different ACPs are used in **fatty acid** and polyketide biosynthesis in *Streptomyces*. We conclude from these data and earlier information that polyketide biosynthesis in *S. glaucescens*, and most likely in other bacteria, involves a multienzyme complex consisting of at least five types of enzymes: acylCoA transferases that load the acyl and 2-carboxyacyl precursors onto the ACP; a .beta.-ketoacyl **synthase** that, along with the acylated ACP, forms the poly-.beta.-ketoacyl intermediates; a poly-.beta.-ketone cyclase that forms carbocyclic structures from the latter intermediates; a .beta.-ketoacyl oxidoreductase that forms .beta.-hydroxyacyl intermediates or reduces ketone groups in fully formed polyketides; and a thioesterase that releases the assembled polyketide from the enzyme.

4/7/4 (Item 1 from file: 35)
 DIALOG(R)File 35:Dissertation Abstracts Online
 (c) 1997 UMI. All rts. reserv.

01194305 ORDER NO: AADD--93876

STRUCTURE AND FUNCTION OF THE CUCUMBER MALATE **SYNTHASE GENE** AND
EXPRESSION DURING **PLANT** DEVELOPMENT (**GENE** EXPRESSION, CUCUMIS
SATIVUS)

Author: GRAHAM, IAN ALEXANDER

Degree: PH.D.

Year: 1989

Corporate Source/Institution: UNIVERSITY OF EDINBURGH (UNITED KINGDOM) (0450)

Source: VOLUME 52/07-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 3406. 157 PAGES

Available from UMI in association with The British Library. Requires signed TDF.

In higher **plants** the glyoxylate cycle is responsible for the net conversion of two molecules of acetyl CoA, derived from β -oxidation of **fatty** acids, into succinate which serves as a substrate for carbohydrate synthesis. This pathway of gluconeogenesis is crucial during germination of fat storing seeds. The glyoxylate cycle enzymes are contained within subcellular organelles termed glyoxysomes. The key glyoxylate cycle enzymes malate **synthase** (MS) and isocitrate lyase are developmentally regulated in that they appear in the lipid storing organs during seed maturation, increase to high levels during germination and thereafter decline to undetectable levels.

In the present work the complete sequences of a full length cDNA clone and a genomic clone encoding the Cucumis sativus MS enzyme have been determined. Putative control regions at the 5' end of the **gene**, three introns, and possible alternative polyadenylation sites at the 3' end have been identified. The deduced amino acid sequence predicts a polypeptide of 64,961 molecular weight, which has 48% identity with that of Escherichia coli. Comparison of the sequence of MS from cucumber with that from E. coli and with other glyoxysomal and peroxisomal enzymes, shows that a conserved C-terminal tripeptide is a common feature of those enzymes imported into microbodies.

In vivo analysis of a 5119 bp fragment, containing the MS **gene** plus 5' and 3' flanking regions, in transgenic Petunia and Nicotiana plumbaginifolia **plants** show that this fragment encodes a functional MS **gene** that is faithfully expressed, both temporally and spatially, in the heterologous host.

Gene fusion studies using the β -glucuronidase (GUS) reporter **gene** indicate that cis-acting elements necessary for transcriptional regulation of the MS **gene** are contained on a 1078 bp promoter fragment that extends from position -1034 to position +44 relative to the start of transcription. Histochemical analysis of reporter **gene** activity reveal that GUS, under the control of the MS promoter, is active in a tissue specific manner in the cotyledons of germinating seedlings

In addition to being expressed in a highly regulated manner during germination, MS also appears to be expressed in senescent cucumber leaves and in petals. The 1078 bp MS promoter fragment also activates transcription of the GUS reporter **gene** in senescent leaves of transgenic N. plumbaginifolia. Possible control mechanisms for MS **gene** expression are discussed.

4/7/5 (Item 2 from file: 35)
DIALOG(R)File 35:Dissertation Abstracts Online
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01120867 ORDER NO: AAD90-25382
PURIFICATION AND CHARACTERIZATION OF ENOYL-ACP REDUCTASE FROM EUGLENA
GRACILIS

Author: TUCKER, MARGIE MCGEE

Degree: PH.D.

Year: 1990

Corporate Source/Institution: EAST TENNESSEE STATE UNIVERSITY (0069)

Enoyl-(acyl-carrier-protein) reductase was purified from the phytoflagellate *Euglena gracilis*. Its purification employed DEAE-Sephacel chromatography, Matrex Orange chromatography, and affinity chromatography using acyl carrier protein (ACP) covalently bound to Sepharose as the affinity ligand. Matrex Orange chromatography resolved two different enoyl-ACP reductases having different characteristics. *Euglena gracilis* appears to resemble higher **plants** in the possession of two isoforms of this enzyme.

Antibodies specific for the cofactor binding site of NADP (H)-requiring dehydrogenases were obtained. They were isolated from a polyclonal population of antibodies directed against yeast glucose-6-phosphate dehydrogenase by affinity chromatography using chicken liver malic enzyme as the affinity ligand. The affinity purified antibodies were covalently bound to Sepharose. Glucose-6-phosphate dehydrogenase and malic enzyme were both bound by the antibody column and were eluted by their cofactor, NADP, identifying the site of recognition of the enzymes by the antibodies as the cofactor binding site. The utility of this antibody affinity column was demonstrated by its ability to bind enoyl-ACP reductase, which was eluted by its cofactor, NADPH.

Preliminary studies of the *E. gracilis* **fatty acid synthase** (FAS) **genes** were undertaken using the plasmid pFAS4 (Witkowski et al., 1987), which contains a cDNA insert to part of the rat liver FAS mRNA and was a gift of Dr. Stuart Smith. The insert was cleaved with KpnI and PstI to generate probes specific for the ketoreductase, ACP, and thioesterase domains of the FAS. DNA from wild type *E. gracilis* and from a mutant, W₁₀BSML, which lacks chloroplast DNA, was subjected to field inversion gel electrophoresis and the DNA alkaline-blotted onto Nylon membranes. Hybridization of the three probes to the DNA was performed; all three probes hybridized to nuclear DNA, but none of the three hybridized to chloroplast DNA. The three probes also hybridized to a band which was neither nuclear nor chloroplast DNA. This DNA, which was larger than the chloroplast genome, may represent *E. gracilis* mitochondrial DNA sequences.

4/7/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
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8590219 EMBASE No: 92266086
Drug development through the genetic engineering of antibiotic-producing microorganisms
Hutchinson C.R.; Borell C.W.; Donovan M.J.; Kato F.; Motamedi H.; Nakayama H.; Otten S.L.; Rubin R.L.; Streicher S.L.; Stutzman-Engwall K.J.; Summers R.G.; Wendt-Pienkowski E.; Wessel W.L.
School of Pharmacy, University of Wisconsin, 425 N. Charter St., Madison, WI 53706 USA
ANN. NEW YORK ACAD. SCI. (USA) , 1991, 646/- (78-93) CODEN: ANYAA
ISSN: 0077-8923
LANGUAGES: English

4/7/7 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
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8326466 EMBASE No: 92004262
Isolation and characterization of *Saccharomyces cerevisiae* mutants resistant to aculeacin A
De Mora J.F.; Gil R.; Sentandreu R.; Herrero E.
Department de Ciencies Mediques Basiques, Facultat de Medicina, Estudi General de Lleida, Rovira Roure 44, 25006 Lleida Spain

ANTIMICROB. AGENTS CHEMOTHER. (USA) , 1991, 35/12 (2596-2601) CODEN:
AMACC ISSN: 0066-4804

LANGUAGES: English SUMMARY LANGUAGES: English

Aculeacin A is a lipopeptide that inhibits beta-glucan synthesis in yeasts. A number of *Saccharomyces cerevisiae* mutants resistant to this antibiotic were isolated, and four loci (ACR1, ACR2, ACR3, and ACR4) whose products are involved in the sensitivity to aculeacin A of yeast cells were defined. Mutants containing mutations in the four loci were also resistant to echinocandin B, another member of this lipopeptide family of antibiotics. In contrast, *acr1*, *acr3*, and *acr4* mutants were resistant to papulacandin B (an antibiotic containing a disaccharide linked to two **fatty** acid chains that also inhibits beta-glucan synthesis), but *acr2* mutants were susceptible to this antibiotic. This result defines common and specific steps in the entry and action of aculeacin A and papulacandin B. The analysis of double mutants revealed an epistatic effect of the *acr2* mutation on the other three mutations. Cell walls of the four different mutants did not show significant alterations in composition with respect to the parental strain, and in vitro glucan **synthase** activity was also unaffected. However, cell surface hydrophobicity in three of the mutants was considerably decreased with respect to the parental strain.

4/7/8 (Item 3 from file: 73)

DIALOG(R)File 73:EMBASE

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8227233 EMBASE No: 91257207

Cloning, characterization, and high-level expression in *Escherichia coli* of the *Saccharopolyspora erythraea* **gene** encoding an acyl carrier protein potentially involved in **fatty** acid biosynthesis

Revill W.P.; Leadlay P.F.

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW United Kingdom

J. BACTERIOL. (USA) , 1991, 173/14 (4379-4385) CODEN: JOBAA ISSN: 0021-9193

LANGUAGES: English

The erythromycin A-producing polyketide **synthase** from the gram-positive bacterium *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) has evident structural similarity to **fatty** acid **synthases**, particularly to the multifunctional **fatty** acid **synthases** found in eukaryotic cells. **Fatty** acid synthesis in *S. erythraea* has previously been proposed to involve a discrete acyl carrier protein (ACP), as in most prokaryotic **fatty** acid **synthases**. We have cloned and sequenced the structural **gene** for this ACP and find that it does encode a discrete small protein. The **gene** lies immediately adjacent to an open reading frame whose **gene** product shows sequence homology to known beta-ketoacyl-ACP **synthases**. A convenient expression system for the *S. erythraea* ACP was obtained by placing the **gene** in the expression vector pT7-7 in *Escherichia coli*. In this system the ACP was efficiently expressed at levels 10 to 20% of total cell protein. The recombinant ACP was active in promoting the synthesis of branched-chain acyl-ACP species by extracts of *S. erythraea*. Electrospray mass spectrometry is shown to be an excellent method for monitoring the efficiency of in vivo posttranslational modification of ACPs.

4/7/9 (Item 4 from file: 73)

DIALOG(R)File 73:EMBASE

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8139552 EMBASE No: 91169325

The pentafunctional *FAS1* **genes** of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are co-linear and considerably longer than previously estimated

Kottig H.; Rottner G.; Beck K.-F.; Schweizer M.; Schweizer E.
Lehrstuhl für Biochemie, Universität Erlangen-Nürnberg, W-8520 Erlangen
Germany, Federal Republic of
MOL. GEN. GENET. (Germany, Federal Republic of) , 1991, 226/1-2 (310-314)
CODEN: MGGEA ISSN: 0026-8925 ADONIS ORDER NUMBER: 002689259100129X
LANGUAGES: English

The **fatty** acid synthetase (FAS) **gene** FAS1 of the
alkane-utilizing yeast *Yarrowia lipolytica* was cloned and sequenced. The
gene is represented by an intron-free reading frame of 6228 bp
encoding a protein of 2076 amino acids and 229980 Da molecular weight. This
protein exhibits a 58% sequence similarity to the corresponding
Saccharomyces cerevisiae FAS beta-subunit. The sequential order of the five
FAS1-encoded enzyme domains, acetyl transferase, enoyl reductase,
dehydratase and malonyl/palmitoyl-transferase, is co-linear in both
organisms. This finding agrees with available evidence that the functional
organization of FAS **genes** is similar in related organisms but differs
considerably between unrelated species. In addition, previously reported
conflicting data concerning the 3' end of *S. cerevisiae* FAS1 were
re-examined by genomic and cDNA sequencing of the relevant portion of the
gene. Thereby, the translational stop codon was shown to lie
considerably downstream of both published termination sites. The *S.*
cerevisiae FAS1 **gene** thus has a corrected length of 6153 bp and
encodes a protein of 2051 amino acids and 228667 Da molecular weight.

4/7/10 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE
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7551232 EMBASE No: 89273405

Analysis of the nucleotide sequence of the *Streptomyces glaucescens* tcmI
genes provides key information about the enzymology of polyketide
antibiotic biosynthesis

Bibb M.J.; Biro S.; Motamedi H.; Collins J.F.; Hutchinson C.R.

John Innes Institute, Norwich United Kingdom

EMBO J. (United Kingdom) , 1989, 8/9 (2727-2736) CODEN: EMJOD ISSN:
0261-4189

LANGUAGES: English

Key information about the biosynthesis of polyketide metabolites had been
uncovered by sequence analysis of the tetracenomycin C polyketide
synthase genes (tcmI) from *Streptomyces glaucescens* GLA.0. The
sequence data revealed the presence of three complete open reading frames
(ORFs). ORF1 and ORF2 appear to be translationally coupled and would encode
proteins containing 426 and 405 amino acids, respectively. The two deduced
proteins are homologous to known beta-ketoacyl **synthases**. ORF3 begins
70 nucleotides after the stop codon of ORF2 and would code for an 83 amino
acid protein with a strong resemblance to known bacterial, animal and
plant acyl-carrier proteins (ACP). The presence of an ACP **gene**

within the tcm **gene** cluster suggests that different ACPs are used in
fatty acid and polyketide biosynthesis in *Streptomyces*. We conclude
from these data and earlier information that polyketide biosynthesis in *S.*
glaucescens, and most likely in other bacteria, involves a multienzyme
complex consisting of at least five types of enzymes: acylCoA transferases
that load the acyl and 2-carboxyacyl precursors onto the ACP; a
beta-ketoacyl **synthase** that, along with the acylated ACP, forms the
poly-beta-ketoacyl intermediates; a poly-beta-ketone cyclase that forms
carbocyclic structures from the latter intermediates; a beta-ketoacyl
oxidoreductase that forms beta-hydroxyacyl intermediates or reduces ketone
groups in fully formed polyketides; and a thioesterase that releases the
assembled polyketide from the enzyme.

4/7/11 (Item 6 from file: 73)
DIALOG(R)File 73:EMBASE
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6042277 EMBASE No: 86037337

Oligonucleotide probes for bacterial acylcarrier protein **genes**

Hale R.S.; Leadlay P.F.

Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW
UNITED KINGDOM

BIOCHIMIE (FRANCE) , 1985, 67/7-8 (835-839) CODEN: BICMB

LANGUAGES: ENGLISH SUMMARY LANGUAGES: FRENCH

Using a recently-introduced rapid manual method, we have synthesized a family of thirty six individual oligonucleotides of unique sequence (18-mers), which correspond to the conserved amino acid sequence, GADSLD, found at the 4'-phosphopantetheine-binding site of the acylcarrier component of bacterial and **plant fatty acid synthases**.

Hybridisation of each of these oligonucleotides to Southern blots of restricted *Streptomyces erythreus* DNA under stringent conditions showed that (i) only two probes hybridised specifically, (ii) neither probe hybridised to more than one sequence, and (iii) each probe apparently recognised a different DNA sequence. In the same synthesis, ninety-two other oligonucleotides (15-18-mers) were also constructed, mostly in yields of 2-10%.

4/7/12 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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06832411 92003699

cDNA cloning and expression of *Brassica napus* enoyl-acyl carrier protein reductase in *Escherichia coli*.

Kater MM; Koningstein GM; Nijkamp HJ; Stuitje AR

Department of Genetics, Vrije Universiteit, Amsterdam, Netherlands.

Plant Mol Biol (NETHERLANDS) Oct 1991, 17 (4) p895-909, ISSN 0167-4412 Journal Code: A60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The onset of storage lipid biosynthesis during seed development in the oilseed crop *Brassica napus* (rape seed) coincides with a drastic qualitative and quantitative change in **fatty acid** composition. During this phase of storage lipid biosynthesis, the enzyme activities of the individual components of the **fatty acid synthase** system increase rapidly. We describe a rapid and simple purification procedure for the plastid-localized NADH-dependent enoyl-acyl carrier protein reductase from developing *B. napus* seed, based on its affinity towards the acyl carrier protein (ACP). The purified protein was N-terminally sequenced and used to raise a potent antibody preparation. Immuno-screening of a seed-specific lambda gt11 cDNA expression library resulted in the isolation of enoyl-ACP reductase cDNA clones. DNA sequence analysis of an apparently full-length cDNA clone revealed that the enoyl-ACP reductase mRNA is translated into a precursor protein with a putative 73 amino acid leader sequence which is removed during the translocation of the protein through the plastid membrane. Expression studies in *Escherichia coli* demonstrated that the full-length cDNA clone encodes the authentic *B. napus* NADH-dependent enoyl-ACP reductase. Characterization of the enoyl-ACP reductase **genes** by Southern blotting shows that the allo-tetraploid *B. napus* contains two pairs of related enoyl-ACP reductase **genes** derived from the two distinct **genes** found in both its ancestors, *Brassica oleracea* and *B. campestris*. Northern blot analysis of enoyl-ACP reductase mRNA steady-state levels during seed development suggests that the increase in enzyme activity during the phase of storage lipid accumulation is regulated at the level of **gene** expression.

4/7/13 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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06735515 91236783

The role of cysteines in polyketide **synthases**. Site-directed mutagenesis of resveratrol and chalcone **synthases**, two key enzymes in different **plant**-specific pathways.

Lanz T; Tropf S; Marner FJ; Schroder J; Schroder G

Institut fur Biologie II, Universitat Freiburg, Federal Republic of Germany.

J Biol Chem (UNITED STATES) May 25 1991, 266 (15) p9971-6, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Resveratrol and chalcone **synthases** are related **plant**-specific polyketide **synthases** that are key enzymes in the biosynthesis of stilbenes and flavonoids, respectively. The stepwise condensing reactions correspond to those in other polyketide and **fatty-acid synthases**. This predicts that the two proteins also contain cysteines that are essential for enzyme activity because they bind the substrates. We exchanged, in both enzymes, all of the 6 conserved cysteines into alanine by site-directed mutagenesis and tested the mutants after expression of the proteins in the Escherichia coli heterologous system. Only cysteine 169 was essential in both enzymes, and inhibitor studies suggest that it is the main target of cerulenin, an antibiotic reacting with the cysteine in the active center of condensing enzymes. Most of the other exchanges led to reduced activities. In two cases, the enzymes responded differently, suggesting that the cysteines at positions 135 and 195 may be involved in the different product specificity of the two enzymes. The sequences surrounding the essential cysteine 169 revealed no similarity to the active sites of condensing enzymes in other polyketide **synthases** and in **fatty acid** biosynthesis. The available data indicate that resveratrol and chalcone **synthases** represent a group of enzymes that evolved independently of other condensing enzymes.

4/7/14 (Item 3 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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06580978 91206987

Molecular genetics of polyketides and its comparison to **fatty acid** biosynthesis.

Hopwood DA; Sherman DH

John Innes Institute, John Innes Centre for Plant Science Research, Norwich, England.

Annu Rev Genet (UNITED STATES) 1990, 24 p37-66, ISSN 0066-4197
Journal Code: GDP

Contract/Grant No.: GM 39784-03, GM, NIGMS

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC
(119 Refs.)

4/7/15 (Item 4 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

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06461263 90234108

Stilbene and chalcone **synthases**: related enzymes with key functions in **plant**-specific pathways.

Schroder J; Schroder G

Institute fur Biologie II, Universitat Freiburg, Bundesrepublik Deutschland.

Z Naturforsch [C] (GERMANY, WEST) Jan-Feb 1990, 45 (1-2) p1-8, ISSN 0341-0382 Journal Code: ACL

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW LITERATURE

Several years of extensive research using the new powerful techniques of molecular biology have enabled the direct comparison of functionally or evolutionarily related **genes** and their products at the nucleotide and amino acid sequence levels. Two types of **synthase** with similar functions are discussed as an interesting example. Stilbene **synthases**, e.g. resveratrol **synthase**, produce the stilbene backbone as a key reaction in the biosynthesis of stilbene-type phytoalexins. Chalcone **synthase** is a key enzyme in the biosynthesis of flavonoids, including certain phytoalexins derived from a 6'-deoxychalcone which is synthesized by cooperation of chalcone **synthase** with a reductase. Resveratrol and chalcone **synthases** utilize the same substrates (4-coumaroyl-CoA and 3 molecules of malonyl-CoA) and catalyze the same condensing type of enzyme reaction (resulting in sequential addition of acetate units via malonyl-CoA), but the products differ in the newly formed ring systems (resveratrol and naringenin chalcone). A comparative analysis of cloned DNA sequences and of the reaction mechanisms indicates that the two enzymes are closely related. It seems likely that the proteins possess a common scaffold for substrate recognition and for the condensing reaction, and that the different folding of an enzyme-bound intermediate prior to closure of the new aromatic ring is responsible for the formation of the different products. The same type of condensing reaction is utilized by the 2-ketoacyl-ACP **synthases** of fatty-acid biosynthesis. However, the available data indicate that these enzymes share little overall homology with either resveratrol or chalcone **synthase**. One exception may be a short amino acid sequence which corresponds to the active center of the condensing reaction in 2-ketoacyl-ACP **synthases**. (41 Refs.)

4/7/16 (Item 1 from file: 10)

DIALOG(R)File 10:AGRICOLA

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3315808 93054958 Holding Library: AGL

Molecular cloning of the **gene(s)** encoding barley beta-ketoacyl-ACP **synthase** I

Kauppinen, S.

London : Portland Press, c1990.

Plant lipid biochemistry, structure and utilization : the proceedings of the Ninth International Symposium on Plant Lipids, held at Wye College, Kent, July 1990 / edited by P.J. Quinn and J.L. Harwood. p. 450-452.

ISBN: 1855780038

DNAL CALL NO: QK898.L56I55 1990

Language: English

Includes references.

Subfile: OTHER FOREIGN;

Document Type: Article

Four cDNA clones encoding subunit II of barley beta-ketoacyl-ACP **synthase** I were isolated using a polymerase chain reaction (PCR) generated product as a probe. Sequencing of a 1822 bp cDNA revealed an open reading frame of 1542 bp coding for a 514 amino acid polypeptide including a transit peptide of at least 87 residues. The mature protein is highly homologous to known bacterial beta-ketoacyl-ACP **synthases**.

4/7/17 (Item 2 from file: 10)

DIALOG(R)File 10:AGRICOLA

(c) format only 1997 Knight-Ridder Info. All rts. reserv.

3315797 93054947 Holding Library: AGL

Stearoyl-ACP desaturase and a beta-ketoacyl-ACP synthetase from developing soybean seeds

Kinney, A.J. Hitz, W.D.; Yadav, N.S.

London : Portland Press, c1990.

Plant lipid biochemistry, structure and utilization : the proceedings of

the Ninth International Symposium on Plant Lipids, held at Wye College, Kent, July 1990 / edited by P.J. Quinn and J.L. Harwood. p. 126-128.

ISBN: 1855780038

DNAL CALL NO: QK898.L56I55 1990

Language: English

Includes references.

Subfile: OTHER FOREIGN;

Document Type: Article

We have purified, to apparent homogeneity, beta-ketoacyl-ACP synthetase I and stearoyl-ACP desaturase from developing soybean seeds. The N-terminal sequences of the synthetase I and the desaturase were determined and used to make a mixture of degenerate oligomers as hybridization probes to screen a developing soybean seed cDNA library. The cDNA sequence encoding the desaturase was identified and the sequence encoding the mature polypeptide expressed in *E. coli* as a fusion protein with glutathione-S-transferase. The fusion protein was purified to near homogeneity in a one step purification using glutathione-sepharose affinity chromatography. Extracts of *E. coli* cells expressing the fusion protein had stearoyl-ACP desaturase activity.

4/7/18 (Item 3 from file: 10)

DIALOG(R)File 10:AGRICOLA

(c) format only 1997 Knight-Ridder Info. All rts. reserv.

3126781 91052056 Holding Library: AGL

Expression of an active spinach acyl carrier protein-I/protein-A **gene** fusion

Beremand, P.D. Elmore, D.D.; Dziejnawska, K.; Guerra, D.J.

ARS, USDA, Northern Regional Research Center, **Plant** Biochemistry and Seed Biosynthesis Research Units, Peoria, IL

Dordrecht : Kluwer Academic Publishers.

Plant molecular biology : an international journal on molecular biology, biochemistry and genetic engineering. Jan 1989. v. 12 (1) p. 95-104.

ISSN: 0167-4412

DNAL CALL NO: QK710.P62

Language: English

Includes references.

Subfile: OTHER FOREIGN;

Document Type: Article

A synthetic **gene** encoding spinach acyl carrier protein I (ACP-I) was fused to a **gene** encoding the Fc-binding portion of staphylococcal protein A. This **gene** fusion, under the control of the lambda P(R) promoter, was expressed at high levels in *Escherichia coli* producing a 42 kDa fusion protein. This fusion protein was phosphopantethenylated in *E. coli*. In vitro the ACP portion of the fusion protein was able to participate in acyl ACP synthetase reactions, **plant** malonyl-CoA:ACP transacylase (MCT) reactions, and **plant fatty** acid synthetase (FAS) reactions. Inhibitory effects of high ACP concentrations on in vitro **plant** FAS were observed with the unfused ACP-I but not with the fusion protein. As with unfused ACP-I, the fusion protein was a poor substrate for *E. coli* FAS reactions. When injected into rabbits, the fusion protein was also able to generate antiserum to spinach ACP-I.

4/7/19 (Item 4 from file: 10)

DIALOG(R)File 10:AGRICOLA

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3100686 91034136 Holding Library: AGL

The pentafunctional FAS1 **genes** of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* are co-linear and considerably longer than previously estimated

Kotting, H. Rottner, G.; Beck, K.F.; Schweizer, M.; Schweizer, E.

Universitat Erlangen-Nurnberg, Erlangen, FRG

Berlin, W. Ger. : Springer International.
M G G : Molecular and general genetics. Apr 1991. v. 226 (1/2) p.
310-314.

ISSN: 0026-8925 CODEN: MGGEAE
DNAL CALL NO: 442.8 Z34
Language: English
Includes references.
Subfile: OTHER FOREIGN;
Document Type: Article

The **fatty** acid synthetase (FAS) **gene** FAS1 of the
alkane-utilizing yeast *Yarrowia lipolytica* was cloned and sequenced. The
gene is represented by an intron-free reading frame of 6228 bp
encoding a protein of 2076 amino acids and 229,980 Da molecular weight.
This protein exhibits a 58% sequence similarity to the corresponding
Saccharomyces cerevisiae FAS beta-subunit. The sequential order of the five
FAS1-encoded enzyme domains, acetyl transferase, enoyl reductase,
dehydratase and malonyl/palmitoyl-transferase, is co-linear in both
organisms. This finding agrees with available evidence that the functional
organization of FAS **genes** is similar in related organisms but differs
considerably between unrelated species. In addition, previously reported
conflicting data concerning the 3' end of *S. cerevisiae* FAS1 were
re-examined by genomic and cDNA sequencing of the relevant portion of the
gene. Thereby, the translational stop codon was shown to lie
considerably downstream of both published termination sites. The *S.*
cerevisiae FAS1 **gene** thus has a corrected length of 6153 bp and
encodes a protein of 2051 amino acids and 228 667 Da molecular weight.

4/7/20 (Item 1 from file: 203)
DIALOG(R)File 203:AGRIS
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1251371 AGRIS No: 90-132636
Functional analysis of the fatty acid synthase gene from *Rattus*
norvegicus
Schweizer, M. (Erlangen Univ. (Germany, F.R.). Inst. fuer Mikrobiologie
und Biochemie); Laux, T.; Takabayashi, K.
43. DGF-Vortragstagung, Hamburg (Germany, F.R.), 1 Oct 1987
Fett, 1988, v. 90(7) p. 263-267
Notes: 6 ill.; 37 ref ISSN: 0931-5985
Language: English Summary Language: German, English
Place of Publication: Germany, F.R.
Document Type: Journal Article, Conference, Summary
Journal Announcement: 1612 Record input by Germany, Federal Republic
of

7/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
(c) 1997 BIOSIS. All rts. reserv.

8140000 BIOSIS Number: 91061000

DEVELOPMENTAL CHANGES IN THE EXPRESSION OF S ACYL FATTY ACID
SYNTHASE THIOESTERASE **GENE** AND LIPID COMPOSITION IN THE
UROPYGIAL GLAND OF MALLARD DUCKS ANAS-PLATYRHYNCHOS

KOLATTUKUDY P E; BOHNET S; SASAKI G; ROGERS L

OHIO STATE BIOTECHNOL. CENT., 206 RIGHTMIRE HALL, 1060 CARMACK RD.,
COLUMBUS, OHIO 43210.

ARCH BIOCHEM BIOPHYS 284 (1). 1991. 201-206. CODEN: ABBIA

Full Journal Title: Archives of Biochemistry and Biophysics

Language: ENGLISH

Developmental changes in the composition of the uropygial gland secretory lipids of the postembryonic mallard ducks (*Anas platyrhynchos*) were determined. During the first 3 weeks after hatching, the composition of the secretory lipids remained constant; the lipids consisted of long-chain **wax** esters composed of a complex mixture of n-, monomethyl, and dimethyl fatty acids esterified to n-C16 and n-C18 fatty alcohols. Afterward, as the ducks began to acquire adult feathers, short-chain **wax** esters composed of 2- and 4-monomethyl fatty acids began to appear with 2-methylhexanoyl and 4-methylhexanol as the major acyl components; esters of short-chain monomethyl fatty acids (1 to 12 carbons) constituted 90% of the lipids when the ducks were 2 months old and had acquired adult plumage. The appearance of the short-chain acids in the acyl portion of the **wax** esters was accompanied by the appearance of S-acyl fatty acid **synthase** thioesterase, which can hydrolytically release short-chain acids from fatty acid **synthase** in the gland. Northern blot analysis showed that the gland-specific thioesterase **gene** transcripts began to appear in the gland only 3 weeks after hatching. The appearance of the transcripts and immunologically detectable thioesterase protein reached maximum levels 2 months after hatching, with the acquisition of the adult plumage. Thus, the developmental changes in lipid composition correlated with the changes in the level of expression of the thioesterase **gene**. Expression of other gland-specific **genes** has been previously found to begin just prior to hatching. The gland-specific thioesterase is the first case of delayed expression of a gland-specific **gene**.

7/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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5236437 BIOSIS Number: 81003744

SUPPRESSION OF A THIOESTERASE **GENE** EXPRESSION AND THE DISAPPEARANCE
OF SHORT CHAIN FATTY-ACIDS IN THE PREEN GLAND OF THE MALLARD DUCK
ANAS-PLATYRHYNCHOS DURING ECLIPSE THE PERIOD FOLLOWING POSTNUPTIAL MOLT

KOLATTUKUDY P E; ROGERS L; FLURKEY W

INSTITUTE OF BIOLOGICAL CHEMISTRY AND BIOCHEMISTRY/BIOPHYSICS PROGRAM,
WASHINGTON STATE UNIVERSITY, PULLMAN, WASHINGTON 99164.

J BIOL CHEM 260 (19). 1985. 10789-10793. CODEN: JBCHA

Full Journal Title: Journal of Biological Chemistry

Language: ENGLISH

Wax esters of short chain acids (monomethyl-C6) constitute the major products of the uropygial gland of mallard ducks. During eclipse, the period (June and July) immediately following postnuptial molt, the

production of short chain acyl groups is severely curtailed and longer chain acyl groups become the dominant components; after this period the composition reverts. These changes in composition were accompanied by corresponding changes in the level of S-acyl fatty acid **synthase** thioesterase activity, and the level of the immunologically detectable amount of this enzyme. In vitro translation of the poly(A+) RNA from the gland produced a 30-kDa protein which cross-reacted with rabbit antibodies prepared against this enzyme. The level of translatable mRNA for the thioesterase in the gland dramatically decreased as the birds went into eclipse and all of these changes reverted when the eclipse period was over. These results strongly suggest that the thioesterase is involved in the production of the short chain fatty acids in vivo and that during eclipse the expression of the thioesterase **gene** is suppressed.

7/7/3 (Item 1 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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04848894 85289269

Suppression of a thioesterase **gene** expression and the disappearance of short chain fatty acids in the preen gland of the mallard duck during eclipse, the period following postnuptial molt.

Kolattukudy PE; Rogers L; Flurkey W

J Biol Chem (UNITED STATES) Sep 5 1985, 260 (19) p10789-93, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM-18278, GM, NIGMS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Wax esters of short chain acids (monomethyl-C6) constitute the major products of the uropygial gland of mallard ducks. During eclipse, the period (June and July) immediately following postnuptial molt, the production of short chain acyl groups is severely curtailed and longer chain acyl groups become the dominant components; after this period the composition reverts. These changes in composition were accompanied by corresponding changes in the level of S-acyl fatty acid **synthase** thioesterase activity, and the level of the immunologically detectable amount of this enzyme. In vitro translation of the poly(A)+ RNA from the gland produced a 30-kDa protein which cross-reacted with rabbit antibodies prepared against this enzyme. The level of translatable mRNA for the thioesterase in the gland dramatically decreased as the birds went into eclipse and all of these changes reverted when the eclipse period was over. These results strongly suggest that the thioesterase is involved in the production of the short chain fatty acids in vivo and that during eclipse the expression of the thioesterase **gene** is suppressed.

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Set	Items	Description
S1	200	SYNTHASE? AND (GENE OR GENES) AND (FATTY OR WAX) AND (PLANT OR PLANTS)
S2	11077447	PY>1991
S3	29	S1 NOT S2
S4	20	RD (unique items)
S5	5	WAX AND SYNTHASE? AND (GENE OR GENES)
S6	5	S5 NOT S2
S7	3	RD (unique items)